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Krill in the Arctic and the Atlantic
Climatic Variability and Adaptive Capacity

Lara Kim Hünerlage

HELMHOLTZ GEMEINSCHAFT

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Titel: Die berühmten Berge „Tre Kroner“ (= Drei Kronen; > 1000 m ü. NN.) gelegen im Osten des hocharktischen Kongsfjords (Königsfjord), W-Spitzbergen. Ihre Namen, Svea, Dana und Nora, stehen für Schweden, Dänemark und Norwegen.
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Cover: The famous mountains „Tre Kroner“ (= Three Crowns; > 1000 m above standard zero) located in the East of the high-Arctic Kongsfjord (King’s Fjord), W-Spitzbergen. Their names, Svea, Dana and Nora, stand for Sweden, Denmark and Norway.
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In memory of my father, Gerd Hünerlage

Dedicated to my family

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Die vorliegende Arbeit ist die im Wesentlichen inhaltlich unveränderte Fassung einer Dissertation, die am Fachbereich Biowissenschaften in der Sektion Funktionelle Ökologie des Alfred-Wegener-Instituts Helmholtz-Zentrum für Polar- und Meeresforschung entstand und im November 2014 dem Fachbereich Biologie an der Fakultät für Mathematik, Informatik und Naturwissenschaften der Universität Hamburg vorgelegt wurde.

CONTENTS

THESIS SUMMARY

Krill are key species in marine food-webs and are distributed in all oceans with 86 species worldwide. They occupy a central role as consumers and as secondary producers forming an important food source for higher trophic levels from fish, squid and sea birds to marine mammals such as seals and whales. A change in regional krill populations would thus have a dramatic impact on the ecosystems of many climate zones.

However, scientific knowledge on the ecophysiology of krill is scarce. The state of science is rather patchy (mainly focused on the charismatic Antarctic krill *Euphausia superba*) and there is no or little data available for an understanding of these key species' ecology with a view to predict ecosystem changes in the oceans.

The present PhD thesis aimed to contribute a broad range of ecophysiological data of abundant and ecologically important krill species from the Atlantic and the Arctic to assess these species' metabolic performance and elucidate their overall adaptation to their respective environments under the constraints of ecosystem variability.

The thesis is composed of five scientific publications (Chapters 2 – 6), which address in detail **a)** the temperature-induced metabolic performance and starvation capacity of the dominant krill species (*Euphausia hanseni*) from the highly productive northern Benguela Current system; **b)** the overwintering physiology, i.e. starvation capacity, of the dominant krill species (*Thysanoessa inermis*) from the high Arctic Kongsfjord; **c)** the energetic condition and trophic relationships of five different euphausiid species recently forming the krill community of the high Arctic Kongsfjord (*T. inermis*, *T. raschii*, *T. longicaudata*, *Meganyctiphanes norvegica* and *Nematoscelis megalops*) including an extended regional comparison to *N. megalops* specimens, which were sampled from the north Atlantic (i.e. the Iceland Basin) and the south Atlantic Benguela Current system; **d)** the temperature-induced metabolic performance of four krill species (*T. inermis*, *T. raschii*, *M. norvegica* and *N. megalops*) from the high Arctic Kongsfjord and **e)** the metabolic and transcriptomic response to temperature increase in the dominant krill species (*T. inermis*) from the high Arctic Kongsfjord.

In order to achieve an appropriate approximation of the single species' physiological condition and adaptive capacity within their respective environments, the particular scientific questions of this thesis were tackled by investigating a variety of key physiological parameters: individual life parameters (stomach fullness, hepatopancreas colour, sex, size and body weight; Publications I – V), species-specific moulting rates (Publications I and II), metabolic rates (i.e. respiration and excretion rates; Publications I, II, IV and V), carbon demand, citrate synthase activity and kinetics (Publication I), body carbon to nitrogen ratios, total protein and lipid contents (Publications I – III), lipid class and fatty acid analyses (Publication III), stable isotope analyses (Publications I – III) and gene expression of the heat shock protein 70 (Publication V).

In the scientific context of **Publication I**, the results physiologically defined the most abundant krill species of the northern Benguela Current system as a true upwelling species. Adult *Euphausia hanseni* were respiratorily well-adapted to strong temperature changes characteristic for this environment (4 – 15°C). The species exhibited a very low lipid content (~ 8 % which is close to the critical limit for survival) pointing to a limited starvation capacity and accordingly, to a "hand-to-mouth" existence in the year-round highly productive Benguela ecosystem.

In contrast to the northern Benguela Current system, polar environments such as the high Arctic Kongsfjord are determined by pronounced seasonality leading to strong variations in primary production. Accordingly, krill species inhabiting this region have to be adapted to another extreme. Here, the arcto-boreal herbivorous *Thysanoessa inermis* dominates the krill

community. The experiments and investigations of **Publication II** on the physiological mechanisms and the allocation of energy resources revealed that *T. inermis* showed a different starvation survival adaptation than krill species from other regions. In contrast to the Antarctic krill *Euphausia superba*, and the subtropical *E. hanseni*, the arcto-boreal species did not reduce metabolism but used its extensive lipid reserves (up to 50 % mainly stored as wax esters, which is quite extreme for euphausiids) and alternative food sources for survival.

The wax ester dominated high lipid content of *T. inermis* results in a high nutritional value of this species for a variety of its predators. However, during the last decades, the Arctic experienced an increased advection of Atlantic waters, which led to a change in krill species composition by carrying characteristic boreal (*Meganyctiphanes norvegica* and *Thysanoessa longicaudata*) as well as subtropical-boreal euphausiids (*Nematoscelis megalops*) into the ecosystem. This recent community shift furnished the scientific context for **Publication III**. To better predict future changes for the Arctic marine food-chain (which may result from the different feeding modes and different nutritional values of the different species), the study centred on a detailed comparison of nutrition and energy storage strategies of five krill species recently found in the high Arctic Kongsfjord.

The results showed remarkable differences between the krill species, which reflected the diverse feeding behaviours and specific adaptations to the environments of their origin. The boreal and the subtropical species appeared more carnivorous, had significantly lower mean lipid contents (29 % and 10 %, respectively) and different energy storage pattern (triacylglycerols and polar lipids, respectively) than the arcto-boreal species. These differences may result in significant implications for the marine ecosystem of the Kongsfjord and the Arctic in general. However, the suggested ecosystemary context depends on each species success to (physiologically) persist within the challenging environment of high latitudes which may be constrained by low starvation capacity due to low energy storage depots and/or metabolic temperature limits.

For that very reason, **Publication IV** aimed to determine the physiological adaptation to ambient temperature changes of the single krill species, which recently altered their specific biogeographic distributions. Metabolic rate measurements served to characterize the species' metabolic reaction to temperature variation, i.e. to reveal their thermal limits of metabolic adaptability, which may help explain population persistence within the respective environments under aspects of climate variability. The *Thysanoessa* spp. appeared more cold-stenotherm than the other krill species: the upper level of respiratory capacity was reached at 12°C. This may explain these species' current arcto-boreal biogeographic distribution and concomitantly, decreasing abundances reported in lower latitudes. In contrast, the temperate-boreal and the subtropical-temperate krill species showed a higher tolerance to temperature changes, which may explain their recent success in northward expansion. This suggestion was further supported by the good overall physiological condition of these species. Accordingly, at least one of the latter species may profit from the increasing "Atlantification" of the Kongsfjord ecosystem due to its superior physiological plasticity.

Publication V aimed to further increase the knowledge on the thermal limit found for the *Thysanoessa* species via molecular approach, i.e. by monitoring the temperature-mediated gene expression of the molecular chaperone 'heat shock protein 70' (= Hsp70) as a proxy for molecular stress. On the example of *T. inermis*, a heat shock experiment at 10°C with subsequent recovery at 4°C resulted in a high level of Hsp70 gene expression during recovery. Accordingly, the specimens had experienced molecular damage during temperature exposure to 10°C which confirmed the findings on the thermal sensitivity of *T. inermis*. Furthermore, the molecular investigation suggests the upper thermal limit of this species to be below 12°C.

In conclusion, based on the diverse analytical perspectives, the present thesis contributed to the understanding of the physiological performance of six ecologically important krill species from two contrasting environments, i.e. the Benguela upwelling zone and the high Arctic Kongsfjord (Spitsbergen). It highlights species-specific adaptations to their areas of origin and discusses consequences for possible future changes in the respective environments and food-webs with emphasis on the ecosystem changes recently found in the high Arctic Kongsfjord.

LIST OF ABBREVIATIONS

ABF	Angola-Benguela-Front
ABT	Arrhenius breakpoint temperature
AC	Angola Current
ATP	Adenosine triphosphate
AMP	Adenosine monophosphate
ANOVA	Analysis of variance
AWI	Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research
AWIPEV	German-French Research Centre Spitsbergen
BMBF	German Federal Ministry of Education and Research
BSA	Bovine albumin serum
cDNA	Complementary deoxyribonucleic acid
C:N-ratio	Carbon to nitrogen ratio
COSYNA	Coastal observation system for Northern and Arctic Seas
CS	Citrate synthase
DW	Dry weight
EC	Enzyme Commission number
ESACW	Eastern South Atlantic Central Water
FFA	Free fatty acids
FW	Fresh weight
GC	Gas chromatography
GENUS	Geochemistry and Ecology of the Namibian Upwelling System
HPLC	High performance liquid chromatography
HPP	Hepatopancreas (colour)
HS	Hornsund fjord
Hsp70	Heat shock protein 70
Hsp70	Messenger ribonucleic acid of heat shock protein 70
HZG	Helmholtz-Centre Geesthacht, Germany

IMP	Intermoult period
J	Joule
K	Kelvin (absolute temperature)
KF	Kongsfjord
K_m	Michaelis-Menten constant
M 87.1b	R/V Meteor leg 87.1b
Mn	*Meganyctiphanes norvegica*
MOCNESS	Multiple opening-closing net and environmental sensing system
mRNA	Messenger ribonucleic acid
MS	Motor ship
MSM 17/3	R/V Maria S. Merian leg 17/3
MUFA	Monounsaturated fatty acids
n	Number of samples / individuals
NaOH	Sodium hydroxide
NBC	Northern Benguela Current
NH_4-N	Ammonium nitrogen
Nm	*Nematoscelis megalops*
O:N-ratio	Oxygen to nitrogen ratio
O_2	Molecular oxygen
OW	Overwintering /overwintered
PC	Phosphatidylcholine
PCR	Polymerase chain reaction
PE	Phosphatidylethanolamine
PERMANOVA	Permutational multivariate analysis of variance
PUFA	Polyunsaturated fatty acids
qPCR	Quantitative real time polymerase chain reaction
Q_{10}	Temperature coefficient
RNA	Ribonucleic acid

ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
R/V	Research vessel
SACW	South Atlantic Central Water
SD	Standard deviation
SDS	Sexual development stage
SEM	Standard error of the mean
SFA	Saturated fatty acids
SIMPER	Similarity percentage analysis
spp.	Species (plural)
SST	Sea surface temperature
STO	Stomach fullness
TAG	Triacylglycerols
Ti	*Thysanoessa inermis*
TI	Trophic level
TL	*Thysanoessa longicaudata*
TpII	Upper pejus temperature
Tr	*Thysanoessa raschii*
U	Enzyme activity unit
WE	Wax ester
$\delta^{13}C$	Ratio of the stable carbon isotopes ^{13}C and ^{12}C
$\delta^{15}N$	Ratio of the stable nitrogen isotopes ^{15}N and ^{14}N

1 GENERAL INTRODUCTION

Krill (or euphausiids) are shrimp-like holoplanktonic crustaceans which are distributed throughout the world's oceans. They appear in substantial numbers and are pivotal components of the pelagic macrozooplankton community occupying a central trophic position in many ecosystems by directly linking primary production to higher trophic levels (e.g. Einarsson 1945, Falk-Petersen et al. 2000, Dalpadado and Mowbray 2013).

In the Antarctic Ocean, more than 150 million tonnes of krill are annually consumed by its predators, i.e. fish, squid, sea birds, seals and whales (Nicol and Endo 1997 and references therein). Additionally, several hundred thousand tonnes of various krill species are commercially harvested for human consumption (direct or as food additive, e.g. http://krilloil.com), the fishing industry (as aquaculture feed) and various other industrial products (e.g. pharmaceuticals, natural colorants, aquarium food, bait for sport fishing; summarized in Nicol and Endo 1997, McBride et al. 2014).

Despite the globally, widespread distribution of krill and their importance in marine ecosystem functioning, scientific knowledge of these species is still scarce. To date a total 86 different krill species have been recorded. However, the state of science is rather patchy and data concentrate mainly on the Antarctic krill *Euphausia superba* – a truly stenothermic species endemic to the Southern Ocean.

The scientific interest on *E. superba*, and krill in general, started during the times of commercial whaling in the early 20th century when this key group was found to be the main prey item of the large baleen whales harvested (Siegel and Atkinson 2009). Since then, most scientific papers and reviews published on euphausiids have been focussed on this one species, the remaining 85 species receiving much less attention (Tarling et al. 2010). Nevertheless, the Norwegian krill *Meganyctiphanes norvegica* and the North Pacific krill *Euphausia pacifica* are probably the best studied euphausiids of the northern hemisphere, due to their high abundance and importance in the fisheries sector (Nicol and Endo 1997).

Krill significantly contribute to the vertical flux of organic matter in the ocean as most krill species perform diel vertical migration (Steinberg et al. 2000, González et al. 2009). Krill principally stay in the deep during the day and migrate to the surface in order to feed during the night (Gibbons 1999). This behaviour is not only a reaction to different light regimes and the avoidance of predators, but also permits these species to remain in favourable water masses and thus, to decrease the effects of advection to e.g. food depleted offshore regions (Barange 1990).

Diel vertical migration implies a physiological challenge, because the species pass through a wide range of different abiotic environmental conditions, such as strong changes in ambient oxygen concentration, salinity and temperature. The latter parameter is a vital factor especially in marine ectotherms as it controls biochemical reactions, determines the species' overall (metabolic) performance and hence, survival and success in individual biogeographic distribution (Pörtner 2001, Pörtner 2002). Furthermore, due to their holoplanktonic lifestyle, krill species are passively subjected to water mass movements, which may transport them into areas with different and possibly unfavourable nutritional conditions (e.g. Verheye and Ekau 2005). Accordingly, krill species have to exhibit diverse physiological adaptations to cope with different environmental factors.

There are no or only few studies on most krill species' special biology and ecophysiology, in particular on individual metabolic performance or chemical composition, although this is important in determining the role of krill in the energy flow of the respective ecosystems (Kim et al. 2010).

The present thesis addresses two different regions, i.e. the subtropical-tropical northern Benguela Current system and the Arctic west coast of Spitsbergen, as these areas are vulnerable to climatic variability and have recently experienced shifts in climatic conditions, ocean currents and/or species composition (e.g. Boyer 2000, Karcher et al. 2003, Falk-Petersen et al. 2007, Ekau et al. 2009, Hutchings et al. 2009, Walczowski et al. 2012). The overall study aimed to regionally compare the specific ecophysiology of six different krill species, which are centred in the functioning of these respective (variable) ecosystems as being key components in the food-web and/or commercially harvested: the subtropical ***Euphausia hanseni***; the subtropical-temperate ***Nematoscelis megalops***; the boreal North Atlantic ***Meganyctiphanes norvegica***; the subarctic-boreal ***Thysanoessa longicaudata***; the arcto-boreal ***Thysanoessa inermis*** and the arcto-boreal ***Thysanoessa raschii*** (Fig. 1.1).

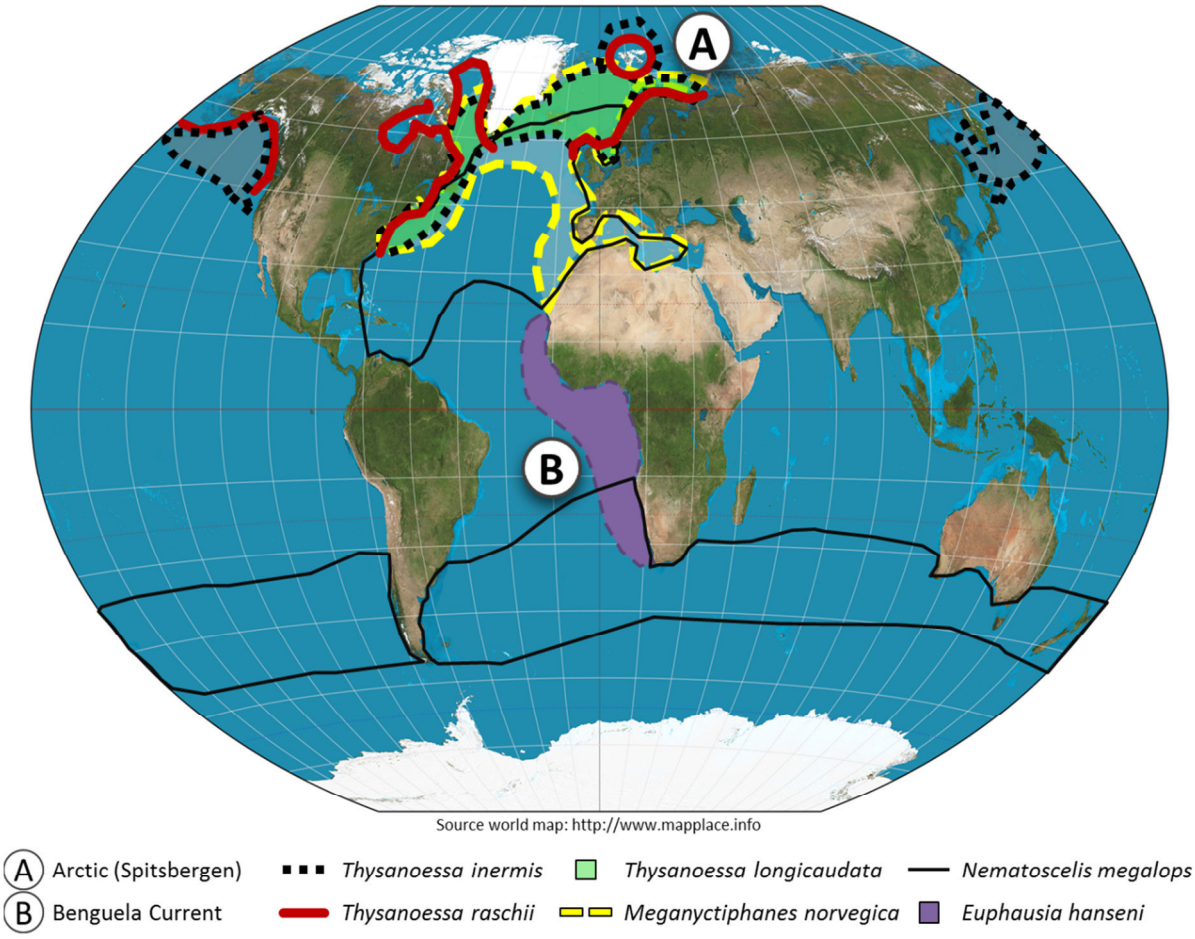

Fig. 1.1 *World map showing the general spatial distribution patterns of the six krill species investigated in the present thesis (Einarsson 1945, Mauchline and Fisher 1969, Gopalakrishnan 1974a, Timofeyev 1993).*

1.1 Krill in the South Atlantic - the Benguela Upwelling Region

Next to the Humboldt Current (Chile and Peru), the California Current (North America) and the Canary Current (north-west of Africa), the Benguela Current belongs to one of the major eastern boundary upwelling currents in the world (Hutchings et al. 2009). Coastal upwelling regions are characterized by equator-ward winds, cool nutrient-rich rising water masses and high plankton biomass. As a consequence, they are economically important fishing grounds, and contribute more than 20 % to the global fisheries (Pauly and Christensen 1995).

The zooplankton community of the Benguela Current system can be dominated by different krill species. Common euphausiid genera are *Euphausia*, *Nematoscelis*, *Nyctiphanes*, *Stylocheiron*, *Thysanopoda* and *Thysanoessa* (Gibbons 1999). Two krill species (*Euphausia hanseni* and *Nematoscelis megalops*; Fig. 1.2) dominate the northern Benguela Current system (Barange and Stuart 1991, Barange et al. 1991). Both species are most abundant at, or close to, the shelf break, i.e. partly sharing one habitat in this upwelling region (Barange et al. 1991).

1.1.1 Euphausia hanseni

The spatial distribution of *E. hanseni* (Fig. 1.2 A) is restricted to the E-Atlantic coast of Africa at both sides of the equator between 26°N and 33°S (Mauchline and Fisher 1969; Fig. 1.1). It is usually found along the shelf-break where it performs extensive diel vertical migration from 0 to 200 and even 1000 m water depth (Barange 1990, Barange and Stuart 1991, Barange and Pillar 1992, Werner and Buchholz 2013). *E. hanseni* is known as a mainly herbivorous filter-feeder above the thermocline where it forms a major nutritional component for economically important fish inhabiting the northern Benguela Current system; e.g. anchovy, hake and horse mackerel (Barange et al. 1991, Barange and Stuart 1991).

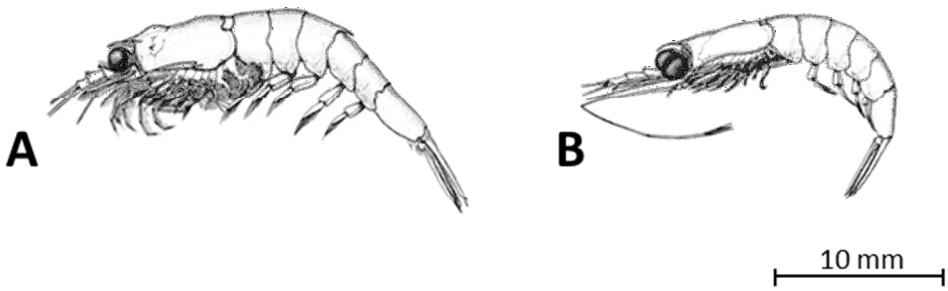

Fig. 1.2 *Adult Euphausia hanseni (A) and Nematoscelis megalops (B). The drawings were obtained from the Marine Species Identification Portal (http://species-identification.org/index.php).*

1.1.2 Nematoscelis megalops

In contrast to *E. hanseni*, *N. megalops* (Fig. 1.2 B) has a broader distribution and can be found at both sides of the equator (Fig. 1.1): in the mid-latitude zones of the subtropical-temperate North Atlantic (10 – 60°N), in the warm-temperate belts of the South Atlantic, the Indian Ocean and the South Pacific (35 – 50°S) and in the Mediterranean Sea (e.g. Gopalakrishnan 1974a). Recently it has been observed to increase in subarctic

regions (Zhukova et al. 2009) and was found up to 79°N in the high Arctic Kongsfjord (Buchholz et al. 2010).

Adult specimens have a mean body size from 22 – 25 mm (Boden et al. 1955). The species is morphologically characterized by its large bi-lobed eyes and its elongated second thoracic appendages which are used for feeding (e.g. Boden et al. 1955, Gopalakrishnan 1974b, Mauchline et al. 1989). These specific appendages are used for active predation on small planktonic metazoans, i.e. allow a form of foraging niche specialization when sharing the habitat with the mainly herbivorous species, *E. hanseni* (Barange et al. 1991).

1.2 Krill in the North Atlantic and the Arctic

The North Atlantic is the habitat of a minimum of 15 krill species from nine different genera (i.e. *Bentheuphausia*, *Euphausia*, *Meganyctiphanes*, *Nematoscelis*, *Nematobrachion*, *Nyctiphanes*, *Stylocheiro*, *Thysanoessa* and *Thysanopoda*; Einarsson 1945). Among these, *Meganyctiphanes norvegica* and the *Thysanoessa* species *T. inermis*, *T. raschii* and *T. longicaudata* dominate the boreal zone of the North Atlantic (Einarsson 1945, Timofeyev 1993).

The presence of krill in the Arctic is mainly referred to the krill community of W-Spitsbergen fjords (e.g. the Hornsund fjord at 77°N and the Kongsfjord at 79°N). In these waters, the dominate euphausiid species are *T. inermis* and *T. raschii* (Einarsson 1945, Timofeyev 1993, Weslawski et al. 2000). In recent years, the high Arctic Kongsfjord has experienced a shift in species composition due to increased input of warm Atlantic waters and subsequent introduction of boreal-temperate (*M. norvegica* and *T. longicaudata*) and even subtropical-temperate species such as *Nematoscelis megalops* (Buchholz et al. 2010; see above)

1.2.1 Meganyctiphanes norvegica

M. norvegica (Fig. 1.3) is the most common krill species found in the North Atlantic and subarctic Atlantic. It can also be found throughout much of the Mediterranean Sea (Einarsson 1945, Mauchline and Fisher 1969; Fig. 1.1) and was recently recorded in the high Arctic Kongsfjord (Buchholz et al. 2010).

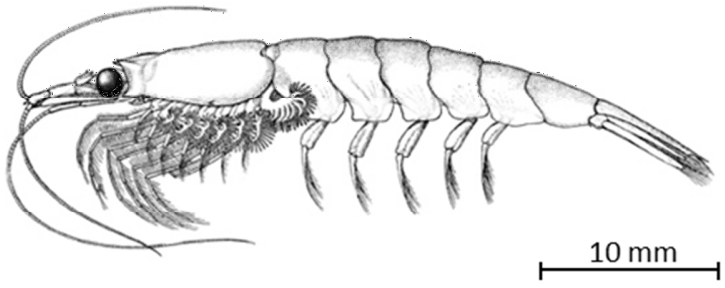

Fig. 1.3 *Adult Meganyctiphanes norvegica. The size of adult specimens ranges from 22 – 50 mm (Tarling et al. 2010). The drawing was obtained from the Marine Species Identification Portal (http://species-identification.org/index.php).*

Next to the Antarctic *E. superba* and some krill species of the genus *Thysanopoda*, *M. norvegica* belongs to one of the largest krill species. It can reach a total body length of 40 – 50 mm (Tarling et al. 2010 and references therein) at a maximum age of about 3 years (Falk-Petersen and Hopkins 1981, Tarling 2010).

The species is known to form huge surface swarms, which can cover an area of more than 100 m² and contain up to 2.2 tonnes of krill per cubic meter (Nicol 1986). Accordingly, it is an attractive food source for many top predators such as the baleen fin whale (*Balaena physalus*), whose stomach can contain more than 500 kg of this species (Brodie et al. 1978). Furthermore, *M. norvegica* is one of the six krill species, which is commercially harvested (Nicol and Endo 1997).

1.2.2 Thysanoessa inermis

The zoogeographic distribution of this arcto-boreal species is restricted to the North Atlantic, North Pacific and the shelf region around Spitsbergen (Fig. 1.1). Nevertheless, it cannot be defined as true Arctic species, as it is unable to successfully reproduce at high latitudes (Timofeyev 1993, 2006, Buchholz et al. 2012). The species originates from the Barents Sea (their major spawning ground at Bear Island 74°N, 19°E) and is continuously advected to the Arctic by the ocean currents. Accordingly, *T. inermis* can be found in all stages (from larvae to adult) in the adjacent waters along the coast of Spitsbergen (Timofeyev 1993).

The morphology off all the three *Thysanoessa* species is similar. However, *T. inermis* is the largest (adult size 25 – 32 mm) and can easily be distinguished by a dorsal spine on the 6th abdominal segment (Boden et al. 1955; Fig. 1.4 A). *T. inermis* is essentially herbivorous and has a life span of 3 – 4 years (Dalpadado and Skjoldal 1996) and is characterized by remarkable lipid contents mainly stored in energy-rich wax esters (Sargent and Falk-Petersen 1981, Saether et al. 1986).

Due to its high lipid content, *T. inermis* is believed to be of great nutritional benefit for higher trophic levels determining the recruitment success, individual growth and overall population condition of many adult fish, seabirds and marine mammals regularly occurring in the Barents Sea and W-Spitsbergen fjords (e.g. Gjøsæter et al. 2002, Dalpadado and Bogstad 2004, Dolgov et al. 2010, Dalpadado and Mowbray 2013, Lydersen 2014). It is commercially harvested in the North Pacific (Nicol and Endo 1997).

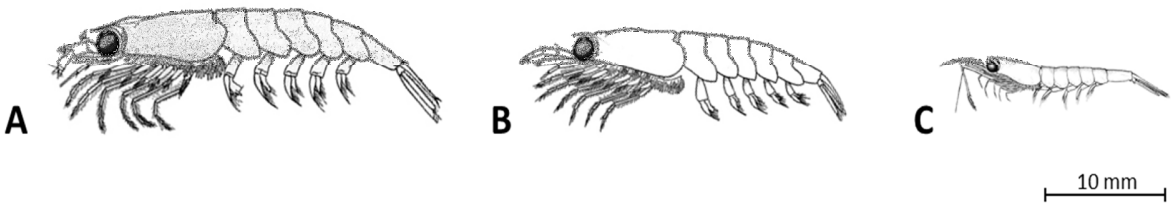

Fig. 1.4 *Adult krill of the genus Thysanoessa: T. inermis (A), T. raschii (B) and T. longicaudata (C). The drawings were obtained from the Marine Species Identification Portal (http://species-identification.org/index.php).*

1.2.3 Thysanoessa raschii

As *T. inermis*, the spatial distribution of *T. raschii* (Fig. 1.4 B) is restricted to the North Atlantic, North Pacific and the shelf region around Spitsbergen, to which it is continuously advected from the Barents Sea (Timofeyev 1993; Fig. 1.1). However it is a more neritic species and mostly found close to the coast above 200 m water depth.

Adult specimens can reach a total body length of 30 mm and a maximum age of 3 years (Falk-Petersen et al. 2000). It can morphologically be distinguished from *T. inermis* by a small denticle which is situated anterior to the middle of the lateral margin (Boden et al. 1955). In contrast to *T. inermis*, *T. raschii* has recently been observed spawning in the high Arctic Kongsfjord at 79°N (Buchholz et al. 2012).

T. raschii is a major food source for many fish, seabirds and marine mammals and belongs to one of the six krill species which are globally harvested for human needs (Nicol and Endo 1997).

1.2.4 Thysanoessa longicaudata

T. longicaudata is a primarily oceanic species and restricted to the boreal North Atlantic (Fig. 1.1). With an adult body length of 10 – 16 mm, it is the smallest of the three *Thysanoessa* species investigated (Fig. 1.4 C). It can be distinguished by its second pair of thoracic legs, which are elongated, thickened and carry setae (bristles) on the last two segments (Einarsson 1945).

T. longicaudata has a life span of up to 2 years and is an important prey for many (juvenile) economically important fish species (Dalpadado and Skjoldal 1996).

1.3 Objectives of the thesis

To date, the ecophysiology of most krill species has received only little attention. The aim of the present thesis is to provide an insight into the physiological performance of six ecologically important krill species from the Atlantic, i.e. from the Benguela upwelling region, and the Arctic – two contrasting regions characterized by distinct climatic variability and thus, environmental conditions.

The focus was set on **a) starvation experiments** as common approaches to uncover the metabolic survival strategy during times of food scarcity, **b) lipid analyses** to elucidate trophic interactions, feeding modes and energetic content and **c) temperature experiments** to reveal the adaptive capacity for population persistence within the respective environments.

The main questions of this dissertation were:

I. **How do krill species, which are adapted to sharply contrasting environments (the highly productive subtropical-tropical Benguela Current system vs. the monopulsed high Arctic Kongsfjord), physiologically respond to periods of food scarcity?** (Chapters 2 and 3)

II. **How do krill species from different environments and recently present in the high Arctic Kongsfjord differ with regard to their energetic condition? Are there implications to be expected for the ecosystem?** (Chapter 4)

III. **How do krill species from different environments metabolically respond to increasing temperatures? Are there species-specific differences in metabolic adaptation which may be related to recent shifts in the species' biogeographic distribution?** (Chapters 2, 5 and 6)

The questions were tackled by the combining of a variety of key physiological parameters: individual life parameters (e.g. stomach fullness, hepatopancreas colour, sex, size and body weight), species-specific moulting rates, metabolic rates (i.e. respiration and excretion rates), carbon demand, citrate synthase activity and kinetics, body carbon to nitrogen ratios, total protein and lipid contents, lipid class and fatty acid analyses, stable isotope analyses and gene expression of the heat shock protein 70.

The scientific knowledge on the ecophysiology of the various krill species will contribute to the assessment of single species population persistence and hence, their food-web role and in the end, of ecosystem functioning.

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RV *Maria S. Merian* during leg MSM 17/3 in the northern Benguela Current

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2 PUBLICATION I

Krill of the northern Benguela Current and the Angola-Benguela frontal zone compared: physiological performance and short-term starvation in *Euphausia hanseni*

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Abstract

Adult *Euphausia hanseni*, keystone and most abundant krill species in the northern Benguela Upwelling area, were sampled during late austral summer in two water masses (northern Benguela Current vs. Angola-Benguela-Front waters) along the Atlantic coast off Namibia. This study investigates the species' physiological performance at different temperatures which it naturally experiences during diel vertical migration as well as its capacity to physiologically adapt to seven consecutive days of starvation. Moulting rates, metabolic rates, carbon demand, total lipid and protein contents, citrate synthase activity and kinetics, C:N ratios and stable isotope ratios served as parameters to estimate the species' physiological condition and adaptive capability within the nutritionally poly-pulsed Benguela upwelling system to cope with short periods of food absence. Moulting rates correlated negatively with temperature. Metabolic rates, adjusted following the Q_{10} rule, declined significantly over the starvation period. Decreasing trends in the other parameters similarly suggest an adaptation to remain metabolically efficient. Ammonium excretion rates, oxygen to nitrogen ratios and stable isotopes showed strong distinctions between regions. Considerable differences were found between regions regarding the nutritional condition of *E. hanseni*. The total lipid content and the physiological reaction to starvation are different from euphausiids from other latitudes and help define *E. hanseni* as a true upwelling organism.

2.1 Introduction

The Benguela Current, located off the southwest coast of Africa, is one of the four major eastern boundary upwelling systems in the world's oceans (Boyer et al. 2000, Hutchings et al. 2009). Owing to its upwelling characteristics (cool, nutrient-rich rising water masses and high phytoplankton biomass), it strongly affects the coastal environment of south-western Africa, and largely determines the yield of local and commercial fisheries (Boyer et al. 2000, Sumaila et al. 2004).

The northern Benguela Current (NBC) is distinctly separated from its southern counterpart by the strong perennial Lüderitz upwelling cell at 27 – 28°S (Boyer et al. 2000), which simultaneously forms the southern border of the NBC. The northern boundary of the NBC consists of a dynamic thermal front of tropical, phytoplankton-poor Angolan water, the Angola Current (AC). The AC and the NBC converge at the Angola-Benguela Front (ABF) (e.g. Meeuwis and Lutjeharms 1990, Kostianoy and Lutjeharms 1999, John et al. 2004, Hutchings et al. 2009). The ABF is characterised by high salinity and strong horizontal temperature gradients with an increase of up to 4°C per degree of latitude (Shannon and

Agenbarg, 1987, Loik et al. 2005). The ABF's presence is determined by the relative strengths of the NBC and the AC and is known as a near-permanent but latitudinally variable feature with a seasonal oscillation of ~ 3° latitude between 10 and 20°S (Meeuwis and Lutjeharms 1990, Boyer et al. 2000). It is located farthest north during austral winter and farthest south during austral summer (Meeuwis and Lutjeharms 1990). On an annual average it can be found between 16°S and 17°S (John et al. 2004, Hutchings et al. 2009).

Oceanic planktonic biotas are passively and continuously transported along with water masses. This transport may subject the plankton to a variable environment and bring them into areas bearing less favourable living conditions (Verheye and Ekau 2005). The Benguela Current system is a nutritionally poly-pulsed and highly stratified environment (Boyer et al. 2000). Herbivorous animals occupying the Benguela Current rely on its seasonal upwelling pulses to create rich phytoplankton patches that supply them as food sources. During late austral summer, the region is characterized by the southernmost reach of the ABF seasonal oscillation and more importantly, minimal upwelling (Hagen et al. 2001). During this season, zooplankton at the northern boundary of the NBC are subjected to unfavourable trophic conditions, brought about by low nutrient concentrations, and may suffer short periods of food deprivation.

The zooplankton biomass of the Benguela Current can at times be dominated (up to 60 %) by euphausiids (krill). Krill species are distributed globally in high abundances and are a keystone species in many marine food webs. They are generally opportunistic omnivores and occupy a central trophic position as secondary producers. Euphausiids forage for both phyto- and mesozooplankton and are thus direct competitors with many fish, particularly zooplanktivorous species and juveniles (Pillar et al. 1992, Sumaila et al. 2004). Many adult fish also rely heavily on krill as a food source (Barange and Stuart 1991, Pillar et al. 1992). In the Benguela Current, at least six euphausiid species are a major source of nutrition to many (commercial) fish, seabirds, seals and whales (Pillar et al. 1992, Gibbons 1997). The physiological condition of the euphausiid populations is therefore important to the maintenance of the whole ecosystem functioning.

Euphausia hanseni is one of the most abundant krill species of the NBC (Olivar and Barange 1990, Barange et al. 1991, Barange and Stuart 1991, Pillar et al. 1992). *Euphausia hanseni*'s distribution is restricted to the Atlantic coast of Africa at both sides of the equator (Mauchline and Fisher 1969), where it is usually found along the shelf-break where it performs extensive diel vertical migrations (Barange 1990, Barange and Stuart 1991, Barange and Pillar 1992, Werner and Buchholz 2013). Consequently, *E. hanseni* encounters a wide range of ambient temperatures which can strongly affect its metabolism and physiological performance (Werner et al. 2012).

Zooplankton species in the challenging northern Benguela upwelling ecosystem must have physiological adaptations, especially to be able to survive under unfavourable trophic conditions. Using *E. hanseni*, this study considers two pertinent questions which arise from this system: (i) How does the metabolism of this key species react to different temperatures? (ii) How does its physiological performance change over short periods of food deprivation?

To date, the ecophysiology of krill, in particular those from the northern Benguela upwelling, has received little attention. The aim of the present study is to investigate a broad range of metabolic parameters [moulting rates, metabolic rates, citrate synthase (CS) activity and efficiency, total lipid and protein content, stable isotope ratios as well as total carbon and nitrogen content] in order to provide an insight into the physiological performance of *E. hanseni* sampled in late austral summer from two adjacent water masses, the NBC and the ABF, characterized by distinct productivities.

2.2 Material and methods

2.2.1 Description of study site

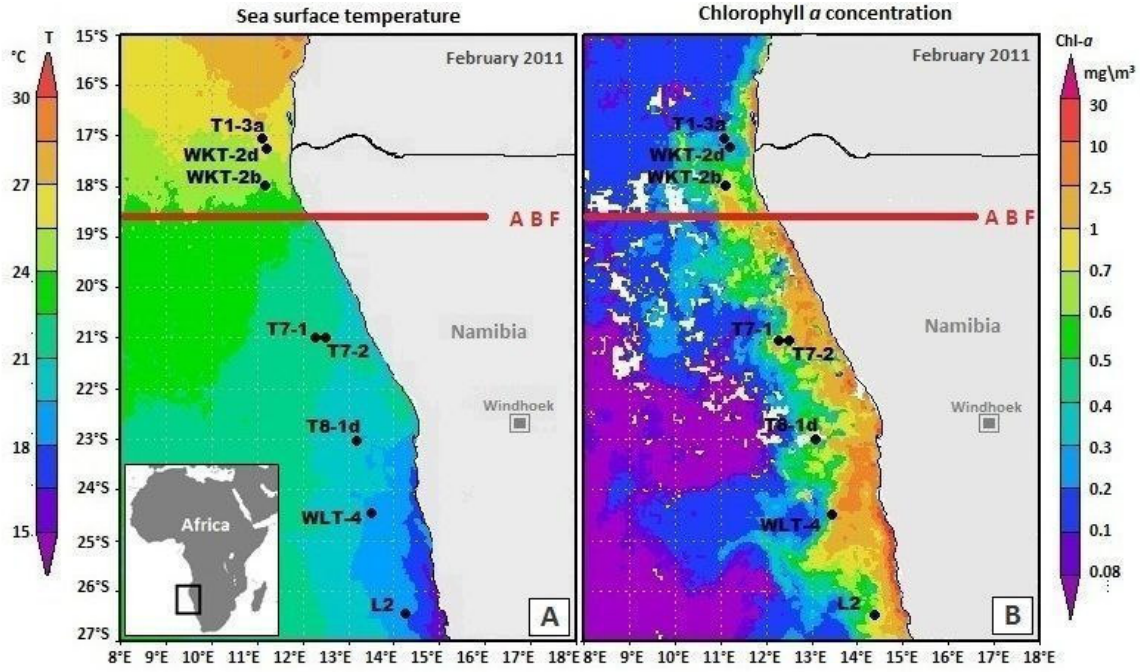

Fig. 2.1 *Satellite images of February averages of (A) sea surface temperature (SST) and (B) chlorophyll a concentration with locations of stations sampled during expedition MSM 17/3 (modified from MODIS-Aqua, http://disc.sci.gsfc.nasa.gov/giovanni). The red line indicates the southern border of Angola-Benguela Front (ABF) at about 18°45'S.*

Eight sampling stations were chosen in the region between 17°15'S, 11°11'E and 26°41'S, 14°26'E (Table 2.1 and Fig. 2.1). The sampling was done in late austral summer 2011 and the southern border of the ABF was at ~ 18°45'S as defined by Ekau and Verheye and Verheye and Ekau (2005). North of this front, the mean sea surface temperature (SST) was > 24°C (Fig. 2.1A).

Maximum chlorophyll *a* concentrations were situated at depths between 30 and 60 m and comprised a component of a large phytoplankton belt reaching up to 120 nautical miles in length off the entire coast of Namibia (Lahajnar et al. 2011). Chlorophyll *a* concentrations at all sites sampled in the NBC (south of 18°45'S) were ≥ 1 mg m^{-3} (Fig. 2.1B), whereas those measured in the ABF (north of 18°45'S) were < 1 mg m^{-3}.

2.2.2 Sample collection

Adult *E. hanseni* were caught on board the German research vessel *Maria S. Merian* (MSM) during leg 17/3 in the coastal upwelling area off the coast of Namibia in late austral summer 2011 (MSM 17/3; 30.01. – 07.03.2011).

Trawls were operated at a speed of two knots using a 1 m^2 MOCNESS (Wiebe et al. 1976) equipped with eight nets (mesh size of 2000 µm) and a soft cod-end net bucket. Hauls were performed during the night when krill were migrating to the surface (50 – 100 m). To facilitate a gentle catch and prevent damaging the animals, hauls were kept as short and shallow as possible.

Captured *E. hanseni* specimens were transferred to aerated aquaria filled with filtered seawater (0.2 µm) and given a 12 h acclimation period at 8°C in the dark, before use in the experiments.

For an estimation of the regional trophic status, stable isotope samples (see chapter "*Biometry and sample preparation*") were taken from acclimated specimens caught at stations T1-8d (NBC) and T1-3a (ABF; Table 2.1; Fig. 2.1).

Table 2.1 *Stations sampled in the northern Benguela Current (NBC) and Angola-Benguela Front (ABF) with information on position and maximum seafloor depth.*

Station	Position	Depth (m)
NBC		
L-2	26°42'S 14°26'E	324
WLT-4	24°31'S 13°42'E	340
T8-1d	23°00'S 13°03'E	413
T7-2	21°05'S 12°52'E	292
T7-1	21°01'S 12°30'E	420
ABF		
WKT2b	18°02'S 11°24'E	416
WKT2d	17°34'S 11°21'E	434
T1-3a	17°16'S 11°11'E	932

2.2.3 Experimental setup

Per region (NBC and ABF), starvation experiments were conducted testing three temperature treatments (5°C, 10°C or 15°C; with 48 krill each). Each experiment lasted 7 days. For technical reasons on board, the starvation experiment at 5°C (ABF) lasted only 6 days.

Krill were slowly acclimated to experimental temperatures over a 2 hour period and then individually placed in aerated 1 L plastic bottles filled with 900 ml filtered sea water (0.2 µm) of a defined treatment temperature. The bottles were kept at experimental temperatures (± 0.5°C, within temperature-controlled rooms) in the dark. Specimens were checked twice daily for mortality and moulting and moulted carapaces were immediately removed from the bottles. The water was exchanged every third day.

Subsamples (n = 3 – 6) of krill were taken daily for respiration rate measurements, after which specimens were frozen in liquid nitrogen and stored at –80°C for later analysis of biometrics, elemental composition, total lipid and protein content, CS activity and CS kinetics. A sample of the experimental water (2 ml) was stored at –80°C for later analysis of excretion rates.

The intermoult period (IMP) for each experiment was calculated according to Tarling et al. (2006). Only the moults occurring within the first 3 days were considered in order to reduce laboratory artefacts (Buchholz 2003, Nicol 2000, Nicol et al. 1992).

2.2.4 Metabolic rate measurements

As a significant expression of overall metabolism, respiration rates were measured by incubating individual *E. hanseni* in special designed respiration chambers: horizontal tubular housings (Perspex; diameter: 20 mm; length: 60 mm) with a total volume of 20 ml; this set-up allowed to measure routine rates (Werner et al. 2012). The chambers were filled with filtered seawater (0.2 µm) at experimental temperature (5, 10 or 15°C) and stored in a water bath in the dark. The oxygen (O_2 mg L^{-1}) concentration inside the chamber was recorded every 15 s using an invasive optical micro sensor system (PreSens GmbH, Germany). At least one chamber per treatment temperature was prepared without an animal to serve as the control. Recording of measurements was stopped as soon as the O_2 concentration in the test chambers reached 60 % of the start concentration. Finally, respiration rates were determined by linear regression model.

Q_{10} values were calculated from respiration measurements according to van't Hoff (van't Hoff 1884). Carbon requirements were calculated from the respiration rates based on the study of Gnaiger (1983) assuming a respiratory quotient of 0.97 for a protein-dominated metabolism [oxygen to nitrogen ratio (O:N) ratio < 24; Ikeda 1974] and 0.72 for a lipid-dominated metabolism (O:N ratio > 24; Ikeda 1974).

As a major end-product of protein catabolism (Jawed 1969), ammonium (NH_4-N) excretion rates under the various treatment temperatures were determined from a 2 ml sample of the respective experimental water at the end of the experiment. The analysis was done photometrically (Perkin Elmer™ Spectrometer Lambda2 with PTP-6, England) following the phenol-hypochlorite method according to Solorzano (Solorzano 1969).

2.2.5 Biometry and sample preparation

Specimens were sexed and their body length measured from the front of the eyes to the tip of the telson under a stereomicroscope, before being weighed to determine the total fresh weight (FW).

The following analyses required the partitioning of specimens into three separate pieces: cephalothorax attached to the first abdominal segment, second abdominal segment alone and lastly, the third and fourth abdominal segments together. All dissections were done on a cooling element to minimize thawing. Stable isotope analyses utilized the muscle of the third and fourth segments without the chitinous exoskeleton. These samples were lyophilized for 24 h and analysed by Agroisolab GmbH (TÜV Rheinland Group; Jülich, Germany). The cephalothorax with the first abdominal segment was lyophilized for 24 h and pestled to powder using a glass tissue grinder. This homogenate was used for CN analysis (1 – 2 mg) as well as for the determination of total lipid (3 – 10 mg) and total protein (1 – 1.5 mg) content in *E. hanseni* specimens from stations T7-2 (NBC) and WKT2b/d (ABF). CS analyses (EC 2.3.3.1) utilized the second abdominal segment without the chitinous exoskeleton.

2.2.6 Biochemical composition

The analyses of C:N ratio, total lipid and total protein content aimed to find possible differences in body composition both between regions and over time of starvation.

CN analysis, as an estimation of the proximate biochemical composition, used aliquots of the pestled cephalothorax and was performed by using an elemental analyser (Euro EA CHNS-O elemental analyser, HEKAtech GmbH, Germany). Acetanilide was used as the standard.

Lipids were extracted from krill homogenate essentially based on the study by Folch et al. (1957). The total lipid content was subsequently determined gravimetrically (Hagen 2000) and expressed as a percent of dry weight (% DW).

Total protein was determined using the Bio-Rad DC Protein Assay (Bio-Rad, Munich, Germany). Bovine albumin serum (BSA) served as the standard. The krill powder was homogenated in 1 ml NaOH (1M) by using an ultrasound sonotrode at ~ 35 % of maximum power (BRANSON SONIFIER® cell disruptor B15, Germany) and incubated for 2 h at 60°C. Afterwards, the homogenate was centrifuged at room temperature for 10 min at 10 000 g, and the supernatant taken for photometrical determination of the total protein content (750 nm; Perkin ElmerTM Spectrometer Lambda2 with PTP-6, England).

2.2.7 Citrate synthase (EC 2.3.3.1)

CS is a key enzyme in metabolism as it plays a central role in the tricarboxylic acid cycle for the supply of energy during aerobic metabolism (Torres and Somero 1988a and 1988b, Buchholz and Saborowski 2000, Meyer et al. 2009). It is often used to reveal physiological adjustments to different environmental conditions (Buchholz and Saborowski 2000, Meyer et al. 2002).

As a proxy for metabolic activity, CS activity was measured based on the study by Stitt (1984). Fresh tissue (second abdominal segment) was homogenized in 500 µl of ice-cold CS buffer (0.05 M Tris-HCL + 0.1 M KCL + 1 mM EDTA; adjusted at room temperature to a pH of 8.0), sonicated on ice at ~ 35 % of maximum power (BRANSON SONIFIER® cell disruptor B15, Germany) and then centrifuged for 10 min at 4°C and 10 000 g. For analysis, 30 µl of supernatant, 30 µl of Acetyl-Coenzyme A (-CoA) solution (6 mM; Milli-Q), 30 µl DTNBA-solution [6 mM 5,5'-dithio-bis(2-nitrobenzoic acid); CS-buffer] and 780 µl of CS-buffer were combined in a glass cuvette and incubated for 5 min at 25°C in the photometer (Perkin Elmer^{TM} Spectrometer Lambda2 with PTP-6, England). The reaction was initiated by adding 30 µl of oxaloacetic acid solution (12 mM; Milli-Q) and monitored continuously for 3 minutes at 412 nm. The activity was both expressed in U mgFW^{-1} and U mgProtein^{-1}. The protein content of the homogenate was determined according to Bradford (1976).

In order to determine the enzyme efficiency of CS, Michaelis-Menten constants (K_m) were measured in specimens from stations T7-2 (NBC) and WKT2b/d (ABF). Measurements were conducted for CS activity towards Acetyl-CoA concentration as described in Saborowski and Buchholz (Saborowski and Buchholz 2002, Vetter 1995a and 1995b). K_m values were calculated using GraphPad Prism5 (GraphPad Software, Inc., USA).

2.2.8 Statistics

A one-way ANOVA with the post-hoc Tukey test was performed in order to test for the influence of temperature on metabolic rates in freshly caught krill (t_0 = unstarved or rather at first day of starvation). A one-way ANOVA with the *post hoc* Dunnett test was performed to detect significant differences between starved (days of starvation two to seven) and non-starved krill (t_0). Additionally, a *post hoc* test for linear trend was used to detect significant trends over time of starvation. Pairwise comparisons were carried out with unpaired t-tests. The significance level was set at $p < 0.05$. All results are given as means

± standard deviation (SD). All statistics were performed using GraphPad Prism5 (GraphPad Software, Inc., USA).

2.3 Results

2.3.1 Hydrography

All stations sampled in the northern Benguela Current (NBC) showed lower water temperatures and salinities throughout the water column compared with the stations investigated in the ABF. In the upper 60 m, the water masses of the ABF were on average 4°C warmer and 2 % more saline than in the NBC and showed a more pronounced surface layer. SSTs of up to 25°C (at 0 – 20 m depth; station WKT2-d) illustrate the strong influence of tropic waters originating from the Angola Gyre (Fig. 2.2).

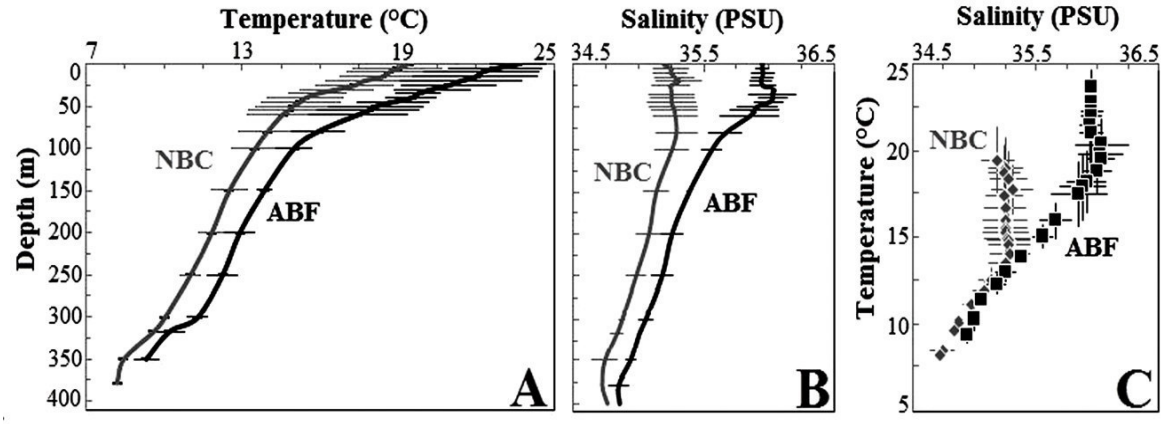

Fig. 2.2 *Depth profiles of temperature (**A**) and salinity (**B**) at the stations sampled in the northern Benguela Current (NBC) and the Angola-Benguela Front (ABF). (**C**) The temperature versus salinity profile indicates two water masses: Eastern South Atlantic Central Water (ESACW) at the NBC and South Atlantic Central Water (SACW) at the ABF. All values are given as means ± SD. Based on MOCNESS data.*

2.3.2 Morphometrics and IMP

During the season studied (late austral summer 2011), adult *E. hanseni* in the NBC were on average longer and heavier than specimens sampled in the ABF (Table 2.2; length: $p < 0.0001$, df = 174 and weight: $p < 0.0001$, df = 176). Proportions of females and males were roughly the same in the starvation experiments at 5°C and 10°C (*E. hanseni* from the NBC), dominated by females in the 5°C and 10°C (from the ABF) and dominated by male specimens in the 15°C experiments (from the NBC and the ABF; see Table 2.2).

The intermoult period (IMP) was negatively related to temperature. A temperature increase from 5°C to 15°C resulted in a 10 day shortening of the IMP. *Euphausia hanseni* sampled from station T7-2 (NBC) deviated from the overall trend at the experimental temperature of 10°C and had a longer IMP of 31.5 d (Table 2.2).

Table 2.2 *Length, fresh weight (FW), sex ratio and intermoult period (IMP) of adult Euphausia hanseni divided by the region of catch (the NBC and the ABF) and the temperature treatment in starvation experiments. Values are given as means ± SD (n = sample size). Sex is given as the ratio of male (m) and female (f) specimens.*

	experimental temperature (°C)	Stations sampled	Length (mm)	FW (mg)	Sex ratio (m:f)	IMP (day)	*n*
E. hanseni **NBC**	5	L2, WLT4,T8-1d	24.9 ± 2.2	113.4 ± 29.9	1 : 1.2	21.0	29
	10	T7-2	23.6 ± 1.4	93.3 ± 18.9	1 : 0.8	31.5	38
	15	T8-1d, T7-1	23.0 ± 1.3	87.0 ± 14.2	1 : 0.4	8.8	45
E. hanseni **ABF**	5	WKT2b/d	22.2 ± 2.0	78.8 ± 21.1	1 : 3.5	18.0	18
	10	WKT2b/d	21.9 ± 2.1	76.2 ± 21.3	1 : 3.0	12.3	24
	15	T1-3a	20.6 ± 1.8	65.0 ± 16.5	1 : 0.6	8.3	24

2.3.4 Elemental and biochemical composition

Nitrogen (N) accounted for ~ 11 % of the total body dry weight (DW), while carbon (C) accounted for ~ 40 % in all specimens sampled (Table 2.3). No change in N or C contents could be detected over the duration of the starvation or between regions. The total body protein content (~ 57 % DW) as well as the total lipid content (~ 8.5 % DW) remained constant over the course of the experiments and were not statistically different between regions (Table 2.3).

Mean stable nitrogen isotope ratios differed significantly between krill from stations sampled in the NBC ($\delta^{15}N$ = 7.05 ± 0.35 ‰) and krill sampled in the ABF ($\delta^{15}N$ = 7.88 ± 0.80 ‰; $p < 0.05$, df = 10; Fig. 2.3). Mean stable carbon isotope ratios ($\delta^{13}C$) also differed significantly ($p < 0.05$, df = 10). Krill from the NBC had a significantly lower mean $\delta^{13}C$ ratio (–19.45 ± 0.84 ‰) than krill from the ABF (–17.88 ± 1.25 ‰; Fig. 2.3).

Table 2.3 *Elemental and biochemical composition of adult Euphausia hanseni (cephalothorax + first abdominal segment) during short-term starvation. Compared are values from first and last days of starvation and region of catch (NBC station T7-2 and ABF station WKT2b/d). Values are given as mean ± SD (n = 6). DW = dry weight, C = body carbon, N = body nitrogen.*

	NBC		**ABF**	
	days of starvation		days of starvation	
	1	7	1	6/7
Elemental composition				
N (% DW)	11.1 ± 0.6	11.3 ± 0.5	11.0 ± 0.5	11.2 ± 0.5
C (% DW)	40.6 ± 1.6	39.7 ± 1.2	39.9 ± 1.2	39.5 ± 1.6
C:N ratio	3.6 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
Biochemical composition				
Total proteins (% DW)	57.3 ± 4.8	58.5 ± 1.7	56.1 ± 0.9	56.7 ± 2.0
Total lipids (% DW)	8.9 ± 2.0	7.9 ± 1.8	8.7 ± 2.5	8.4 ± 2.3

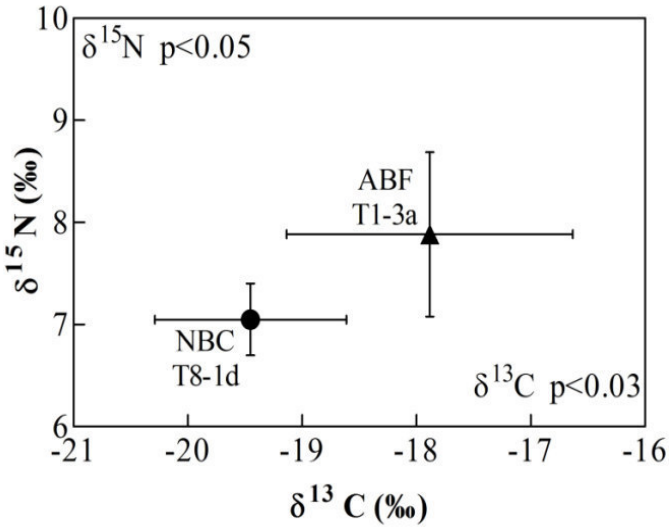

Fig. 2.3 *Stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) measured in the third and fourth abdominal segments of freshly caught adult Euphausia hanseni. Specimens from northern Benguela Current (NBC station T8-1d) and Angola-Benguela Front (ABF station T1-3a) are compared. Values are given as means ± SD (n = 6).*

2.3.5 Metabolic rates

Respiration rates were positively related to ambient temperature and increased significantly in both regions from an average of ~ 7 µmol O_2 gFW^{-1} h^{-1} at 5°C to an average of ~ 18 µmol O_2 gFW^{-1} h^{-1} at 15°C (Fig. 2.4; NBC: $p < 0.01$, F = 9.36, df = 11; ABF: $p < 0.05$, F = 5.33, df = 8). There were no statistical significant differences between regions. The relationship between temperature increase and respiration rates was best described by exponential regression ($y = y_0 e^{kx}$; NBC: $y_0 = 5.00$, k = 0.009, $r^2 = 0.66$; ABF: $y_0 = 3.80$, k = 0.10, $r^2 = 0.63$). Q_{10} values calculated between 5 and 15°C were 2.44 for the NBC and 2.70 for the ABF (Fig. 2.4).

Over the course of the experimental starvation, all krill but those in the 5°C temperature treatments showed a decrease in mean respiration rates (Fig. 2.5). By the end of the starvation period, respiration rates had fallen by ~ 44 % in the NBC krill ($p < 0.01$, F = 4.71, df = 36) and 41 % in the ABF krill ($p < 0.001$, F = 8.74, df = 22) exposed to the 10°C treatment. A similar but greater reduction was recorded in the 15°C experiments with a decline by ~ 53 % in the NBC krill ($p < 0.01$, F = 4.22, df = 42) and 70 % in the ABF krill ($p < 0.05$, F = 2.10, df = 24) (Fig. 2.5).

Corresponding to the change in respiration rates, the daily carbon demands (% C day^{-1}) were positively and significantly related to temperature in both regions (Fig. 2.6; NBC: $p < 0.01$, F = 9.38, df = 11; ABF: $p < 0.05$, F = 7.23, df = 9). At the beginning of the starvation experiments, the mean carbon requirements of krill from the NBC (1.9 ± 0.4 % C day^{-1} at 5°C, 3.5 ± 1.2 % C day^{-1} at 10°C and 5.2 ± 0.5 % C day^{-1} at 15°C) were higher than those of krill from the ABF (1.4 ± 0.4 % C day^{-1} at 5°C, 2.0 ± 0.4 % C day^{-1} at 10°C and 3.5 ± 1.3 % C day^{-1} at 15°C), although not significantly different (Fig. 2.6).

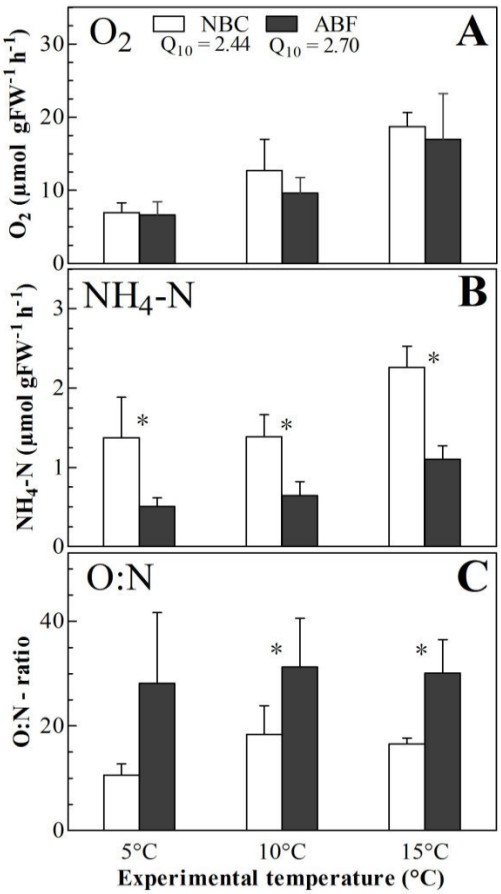

Fig. 2.4 *Metabolic state on the first day of starvation: oxygen consumption (**A**), ammonium excretion (**B**) and atomic O:N ratio (**C**) of adult Euphausia hanseni at 5, 10 and 15°C compared in two regions: northern Benguela Current (NBC) and Angola-Benguela Front (ABF). Values are given as means SD (n = 3 – 6). *Significant differences between regions.*

Similar results were found at the end of the starvation period; krill from the NBC had a higher carbon demand (on average ~ 2.0 % C day^{-1}) than krill from the ABF (on average ~ 1.3 % C day^{-1}). Over the duration of starvation, mean carbon requirements decreased in krill from both regions at all temperature treatments (except for krill from the NBC held at 5°C; Fig. 2.6). The decrease was significant in krill from the NBC held at 10 and 15°C (10°C: $p < 0.05$, df = 5; 15°C: $p < 0.01$, df = 12).

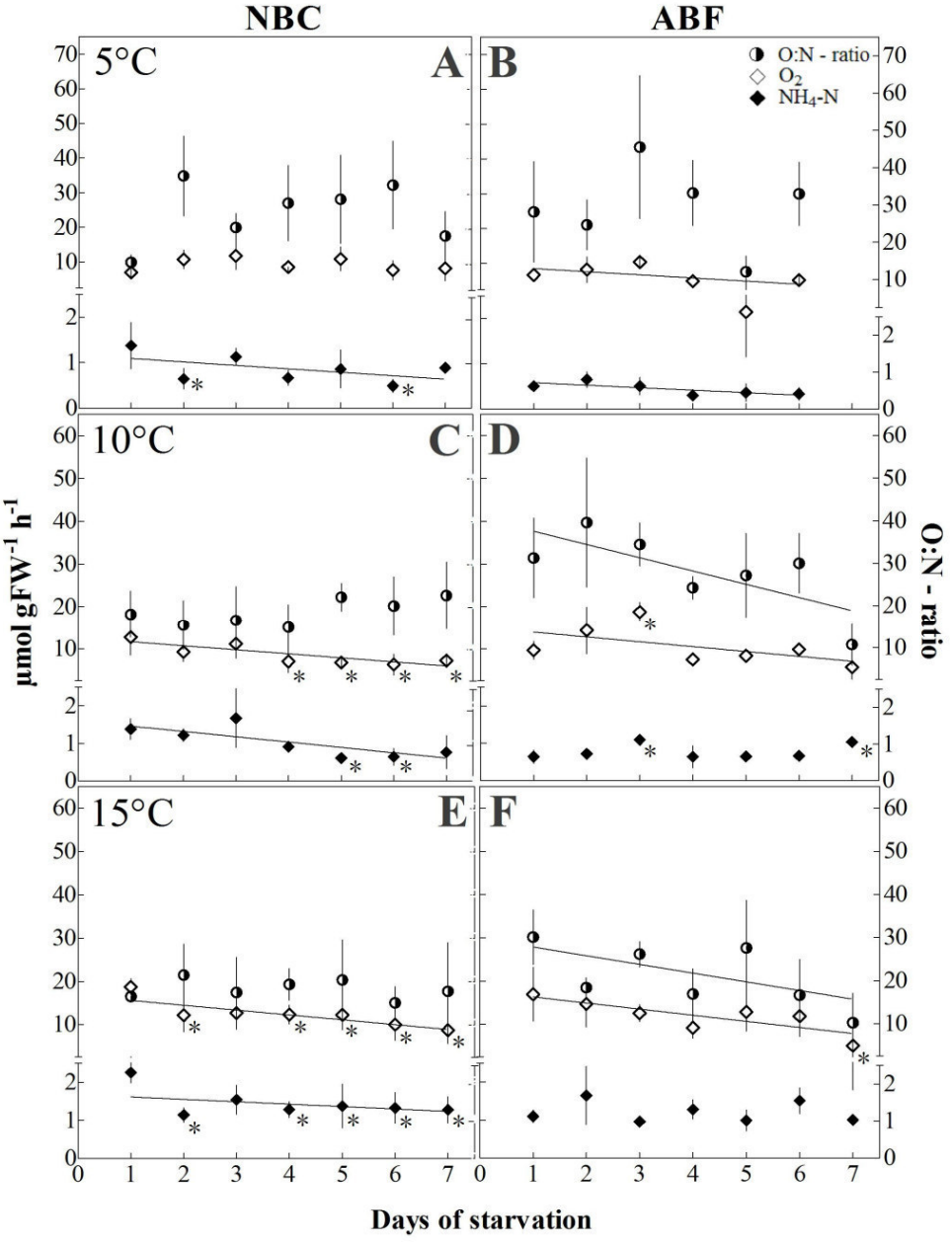

Fig. 2.5 *Oxygen consumption (O_2), ammonium excretion (NH_4-N) and atomic O:N ratio of adult Euphausia hanseni during short-term starvation experiments at 5, 10 and 15°C compared in two regions [(**A**,**C** and **E**): northern Benguela Current (NBC); (**B**, **D** and **F**): Angola-Benguela Front (ABF)]. Values are given as means ± SD (if not visible, SD are covered by the symbol; n = 3 – 12). Lines indicate significant trends over time of starvation. *Significant differences from the first day of starvation.*

Ammonium excretion rates (NH_4-N) were positively related to the ambient temperature (Fig. 2.4; NBC: $p < 0.05$, F = 7.52, df = 11; ABF: $p < 0.01$, F = 12.04, df = 8). Significant differences were found between krill caught in the NBC and the ABF (5°C: $p < 0.05$, df = 4; 10°C: $p < 0.01$, df = 7; 15°C: $p < 0.01$, df = 4). With an average of 1.4 ± 0.5 µmol NH_4-N gFW^{-1} h^{-1} at 5°C and 2.3 ± 0.3 µmol NH_4-N gFW^{-1} h^{-1} at 15°C, the excretion rates of krill from the NBC were more than double compared with the mean rates from krill caught in the ABF (0.5 ± 0.1 µmol NH_4-N gFW^{-1} h^{-1} at 5°C and 1.1 ± 0.2 µmol NH_4-N gFW^{-1} h^{-1} at 15°C). During the starvation period, the excretion rates decreased significantly in all temperature treatments conducted with specimens from the NBC (5°C: $p < 0.05$, F = 3.21, df = 24; 10°C: $p < 0.001$, F = 5.79, df = 34; 15°C: $p < 0.01$, F = 3.71, df = 43). In contrast, the excretion rates of specimens sampled from the ABF remained stable throughout the experiments with mean rates fluctuating around 0.5 µmol NH_4-N gFW^{-1} h^{-1} at 5°C, 0.8 µmol NH_4-N gFW^{-1} h^{-1} at 10°C and 1.2 µmol NH_4-N gFW^{-1} h^{-1} at 15°C (Fig. 2.5).

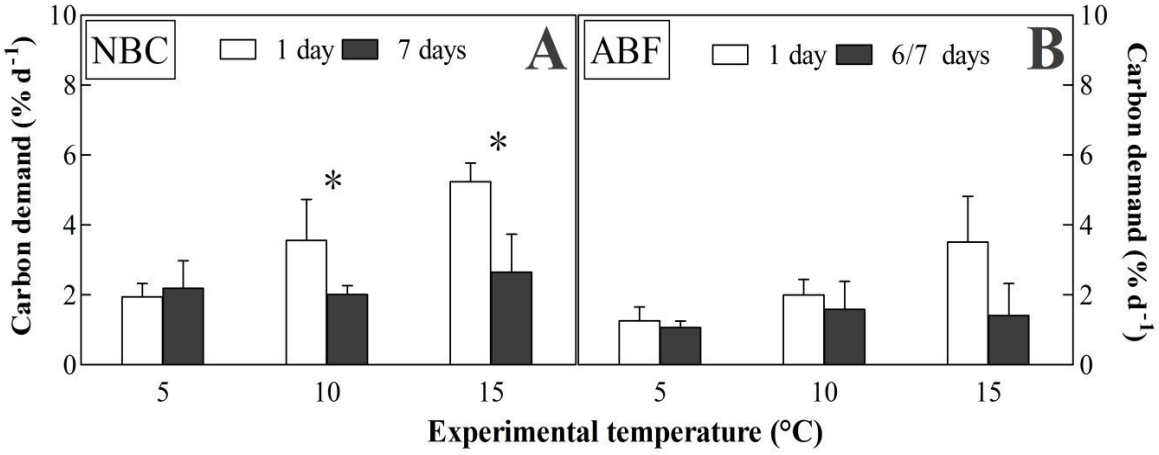

Fig. 2.6 *Carbon demand of adult Euphausia hanseni during short-term starvation experiments at 5, 10 and 15°C compared in two regions: (A) northern Benguela Current (NBC); (B) Angola-Benguela Front (ABF). Values are given as means ± SD (n = 3 – 11). *Significant differences against the first day of starvation.*

The atomic O:N ratio, derived from the measurements of respiration (O) and excretion (N) rates as an indication for the substrate metabolized [lipid O:N > 24; protein O:N < 24 (Ikeda 1974)], was not related to temperature but was significantly different between regions at 10 and 15°C (Fig. 2.4; 10°C: $p < 0.05$, df = 7; 15°C: $p < 0.05$, df = 4). In the NBC, the O:N ratio ranged on average from 10.6 to 18.4 compared with higher mean ratios of up to 31.3 (10°C) in the ABF (Fig. 2.4). During short-term starvation, the atomic O:N ratio did not change in krill sampled from the NBC across all temperature treatments, or in krill from the ABF held at 5°C (Fig. 2.5). At 10 and 15°C, specimens from the ABF showed a significant decrease in O:N ratios to values < 24 indicating a change in the substrate metabolized. In the 15°C treatment, the mean O:N ratio decreased by ~ 65 % over the 7-day period ($p < 0.05$, F = 2.88, df = 21).

2.3.6 Citrate synthase

In all experimental temperatures, mean specific CS activities decreased over the period of starvation for krill from both regions (Table 2.4). The decrease was significant in krill from the NBC tested at 15°C ($p < 0.01$, df = 10). The only exception was found in krill from the NBC held at 10°C, in which the CS activity slightly increased.

Table 2.4 *Citrate synthase (CS) activity at 25°C in U gProtein^{-1}. Numbers in brackets give the activity in U gFW^{-1}. Compared are days of starvation at experimental temperatures between regions sampled (the NBC and the ABF). Values are given as means ± SD (n = 6). *Indicates significant difference over time of starvation.*

	Days of starvation					
	5°C		10°C		15°C	
	1	6/7	1	7	1	7
E. hanseni **NBC**	48.5 ± 24.9 (4.0 ± 0.5)	44.4 ± 23.6 (4.5 ± 0.1)	21.2 ± 4.3 (2.9 ± 0.8)	26.4 ± 9.8 (3.5 ± 0.7)	31.3 ± 4.8 (3.6 ± 0.7)	21.8 ± 5.4* (3.2 ± 0.8)
E. hanseni **ABF**	31.5 ± 8.2 (3.8 ± 1.0)	27.9 ± 11.1 (2.5 ± 0.7)	39.2 ± 12.2 (2.9 ± 0.5)	34.0 ± 11.4 (3.8 ± 0.8)	25.4 ± 16.7 (4.1 ± 1.4)	18.6 ± 7.2 (1.4 ± 0.7*)

While the mean CS activity decreased over the period of starvation, its enzyme efficiency increased as expressed in the decrease of mean Michaelis constants (K_m) of CS with time of starvation in specimens of both regions (Fig. 2.7). The K_m values of krill sampled in the NBC were significantly higher compared with the values of krill caught in the ABF (1 day: $p < 0.05$, df = 9; 7 day: $p < 0.05$, df = 10).

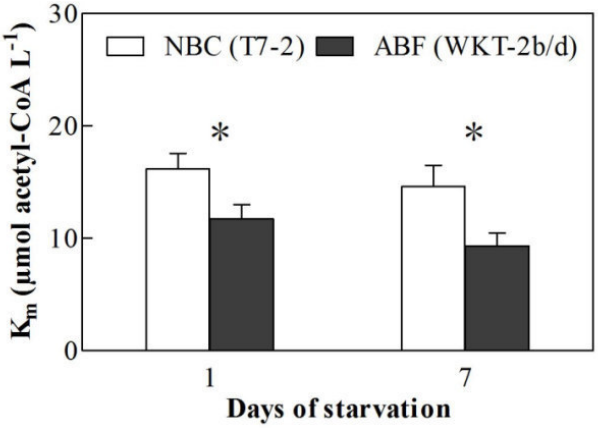

Fig. 2.7 *Michealis-Menten constants (K_m) of citrate synthase (CS) at 25°C. Days of starvation and regions sampled (NBC station T7-2 and ABF station WKT2b/d) are compared. Values are given as mean ± SD (n = 6). *Significant difference between regions.*

2.4 Discussion

2.4.1 Metabolic rates

Overall, our study revealed no regional differences in oxygen consumption rates of *E. hanseni* but indicated a strong relationship between respiration rate and temperature in both regions in agreement with previous studies on krill species of other latitudes (e.g. Saborowski et al. 2002).

The rates increased exponentially with increasing temperatures following the Q_{10} rule (van't Hoff 1884). Q_{10} values of 2.44 (specimens from the NBC) and 2.70 (specimens from the ABF) are consistent with values measured on other krill species (e.g. Hirche 1984, Saborowski et al. 2000, Saborowski et al. 2002) and lie within the common biological range between 2 and 3 (Hirche 1984, Montagnes et al. 2003). Accordingly, the temperature influence on the respiration (physiological reaction) of *E. hanseni* complies with other krill species and corresponds to the conclusions of Saborowski et al. (2000) as it shows the adaptive disability of euphausiids to physiologically compensate for short temperature effects.

Like the oxygen consumption, the ammonium excretion was positively related to the ambient water temperatures but in contrast, strong distinctions between regions were observed at all experimental temperatures. The mean excretion rates of krill sampled from the ABF were significantly lower compared with krill from the NBC. Excretion rates are closely correlated with food ingestion as observed in copepods (Corner et al. 1965) and *M. norvegica* (Saborowski et al. 2002); high phytoplankton concentrations are correlated with high ammonium excretion rates, whereas the absence of food leads to a decrease in excretion. Accordingly, the difference in ammonium excretion rates would be best explained by the lack of sufficient phytoplankton concentration in the region north of 18°46'S, which was strongly influenced by the intrusion of the warm AC during February 2011. Fig. 2.1 shows the chlorophyll *a* concentration as an indication of phytoplankton biomass. The highest chlorophyll *a* concentrations were found in the NBC, i.e. the area between station L2 and the ABF. Furthermore, the zooplankton biomass available for consumption by krill (< 5 mm) was up to 3-fold higher in the NBC compared with the ABF (pers. comm. B. Martin, IHF Hamburg, Germany). Both support the notion that specimens from the NBC were exposed to better nutritional conditions and thus had higher ammonium excretion rates than specimens from the ABF.

Atomic O:N ratios provide a good indication of the diet/substrate metabolized and was therefore used in this study to compare the nutritional condition of specimens between regions and temperature treatments. The ratio is derived from respiration and excretion rates, and according to Ikeda (1974) an O:N ratio of 24 occurs when the rates of protein and lipid metabolism are equal. A ratio < 24 indicates the predominant catabolism of protein. Conversely, the preferential use of lipids is indicated by an atomic O:N ratio > 24 (Ikeda 1974). The lowest ratios were found in *E. hanseni* from the NBC (10 – 18), significantly lower than those from the ABF (28 – 31). Accordingly, specimens from the NBC were characterized by a protein-dominated metabolism (even pure protein metabolism according to Mayzaud and Conover (1988) whereas specimens from the ABF were characterized by a lipid-orientated metabolism. Generally, euphausiids are opportunistic omnivorous, but most of them, like *E. hanseni*, show predominant herbivorous feeding if conditions are optimal (Barange et al. 1991). Hence, the lipid metabolism in the animals from the ABF points to a dietary shift from preferentially herbivorous to carnivorous feeding, which may indicate a deterioration of the trophic environment for krill in this area.

2.4.2 Elemental and biochemical composition

The analysis of elemental body composition in terms of carbon (C) and nitrogen (N) is commonly used to estimate the relative contents of lipids against proteins (Anger and Harms 1990). In our study it was primarily used as a proxy for differences and changes in body composition between the regions and over short periods of starvation respectively. The mean C:N mass ratio, representing the ratio of lipid to protein content, of ~ 3.6 remained constant in all *E. hanseni* specimens during starvation and was not statistically different between regions. This ratio resembles findings in other euphausiids with low lipid content (Stuart 1986, Meyer and Oettl 2005, Auerswald et al. 2009).

In our *E. hanseni* specimens, lipid and protein contents were not statistically different between all animals sampled. The total lipid content of ~ 8 % DW found within all krill samples from the Benguela upwelling sites (both the ABF and the NBC) is similar to another krill species inhabiting the same region, *Nematoscelis megalops*, with a total lipid content ranging from 7.3 – 11.3 % DW^{-1} (Cartes 2010), but is notably lower than that of krill species found in other latitudes, i.e. temperate and polar regions (Falk-Petersen et al. 1981, Hagen and Kattner 1998, Mayzaud et al. 1999, Hagen et al. 2001, Atkinson et al. 2002, Ju and Harvey 2004, Meyer et al. 2010). This discrepancy may be explained by the fact that these other latitudes are characterized by long periods of food absence – compared with short periods prevailing in the nutritionally poly-pulsed Benguela upwelling system. Therefore, krill species from higher latitudes can accumulate very high lipid contents while storing lipids for overwintering (Lee et al. 2006).

According to Hagen et al. (2001), a lipid content of 5 % DW^{-1} is the lowest limit for survival as it is the essential value for the functioning of membranes. Accordingly, the low total lipid content of about 8 % DW^{-1} points to a “hand-to-mouth” existence of *E. hanseni* inhabiting the NBC. It suggests that the species is adapted to regular upwelling pulses with only short periods of food absence. Hence, *E. hanseni* is well adapted to poly-pulsed upwelling environments with no need to accumulate large stores of lipids to survive. It likely directly puts gained energy from nutrition into fast growth and reproduction.

2.4.3 Stable isotopes

Stable isotopic signals result from dietary input, i.e. the food source ingested (Frazer et al. 1997). Analyses are commonly performed with regard to stable nitrogen ($\delta^{15}N$) and stable carbon ($\delta^{13}C$) isotopes in order to determine the trophic position (out of $\delta^{15}N$) and the foraging habitat (out of $\delta^{13}C$) of a species (Jaeger et al. 2010). Furthermore, it can be used to estimate the long-term prevailing trophic conditions that an organism lives within (Fry and Arnold 1982, Tieszen et al. 1983, Båmstedt et al. 2000).

In our approach, stable isotope analysis was performed to estimate the difference in prevailing trophic conditions surrounding krill from both regions: the NBC and the ABF. The analysis showed significant distinctions: *E. hanseni* sampled in the ABF had both higher $\delta^{15}N$ and $\delta^{13}C$ contents compared with animals sampled in the NBC. These results again support the hypothesis that krill from the ABF partake in a more carnivorous diet (higher trophic position due to higher $\delta^{15}N$) than those from the NBC. It also indicates that the krill populations from both regions were already exposed to their different environmental conditions for longer times (Fry and Arnold 1982, Tieszen et al. 1983, Båmstedt et al. 2000).

2.4.4 Citrate synthase

The specific enzyme activity of CS of *E. hanseni* showed no significant differences between any specimens, i.e. temperature treatments or regions investigated. Nevertheless, significant differences between regions were found with respect to CS efficiency (Michealis-Menten constants K_m). Animals sampled from the ABF showed significantly lower K_m values, i.e. had a higher CS efficiency compared with specimens sampled from the NBC. The enzyme efficiency of CS provides an indication of the nutritional and metabolic conditions prevailing for krill sampled from different regions. CS efficiency increases during unfavourable food conditions in order to compensate for a metabolic reduction (Buchholz and Saborowski 2000, Saborowski and Buchholz 2002). These findings support the assumptions of less favourable food conditions prevailing for *E. hanseni* in the ABF or even suggest that these animals may have already been starved before capture.

2.4.5 Intermoult period

Analysis of the IMP of *E. hanseni* did not detect regional differences but found that IMP is related to ambient temperature as the moult interval decreased with increasing temperature similar to other euphausiids, particularly *Euphausia superba* (Buchholz 2003). The only exception appeared in specimens originating from the NBC and held under the 10°C treatment. Here, the IMP was determined to be 31.5 days, longest out of the krill samples from all treatments and regions. This potential outlier may be explained through moult synchronicity. Favourable food conditions such as rich plankton patches immediately initiate somatic growth (Buchholz 1991), which leads to synchronous moulting within krill swarms. Moult processes are accelerated due to fast simultaneous accumulation of energy reserves (Buchholz 2003). These specimens were exclusively caught at station T7-2, located near the area of highest phytoplankton concentration (Fig. 2.1). Therefore, these animals may have taken advantage of the nutritional conditions and had completed their moult cycle synchronously shortly before they were employed in the experiment. Such a scenario would result in the calculation of an artificially long IMP.

The IMPs determined for *E. hanseni* in this study resembled values from previous studies on other krill species kept at similar temperatures (Stuart and Pillar 1988, Couzin-Roudy and Buchholz 1999, Couzin-Roudy et al. 2004, Shaw et al. 2010). This consistency and comparability of IMPs with other species is therefore encouraging, and urges IMP to be used more often in the future to calculate krill productivity through growth.

2.4.6 Physiological changes during short-term starvation

Seven days of starvation significantly affected the metabolism of *E. hanseni* from both regions, the NBC and the ABF. While the strength of the starvation effect differed between the regions with regard to oxygen uptake, ammonium excretion, atomic O:N ratio, carbon-demand and enzyme efficiency of CS, it did not alter the total lipid and total protein contents within the krill.

In all temperature treatments, specimens responded to starvation with a continuous decrease in oxygen consumption by 40 % – 70 % from the first to the last days of starvation while showing no additional effect caused by the incubation temperature. The only exceptions were found in animals from the 5°C experiments, in which the low temperature appeared to compensate for the effect of starvation due to a temperature-dependent reduction in the metabolic rate. Generally, the decline of oxygen consumption rates during starvation is consistent with starvation studies on Antarctic krill

(Meyer and Oettl 2005, Auerswald et al. 2009). Comparing both regions, *E. hanseni* from the ABF reacted more sensitively to food deprivation in terms of oxygen consumption as it displayed the strongest decrease (by 70 %) in the 15°C treatment. This observation might additionally reflect the worse nutritional/physiological condition of krill from the ABF.

Such implications could also explain the change in ammonium excretion rates during 7 days of starvation and also underline the trophic difference between the NBC and the ABF: the excretion rates in krill sampled in the NBC showed a continuous decrease over the period of starvation, whereas rates from the ABF remained constant from the beginning. This may be the consequence of the distinct nutritional situations prevailing in the NBC and the ABF as the specimens of the ABF may have already been exposed to food depletion before capture. In turn, this also points to more favourable food conditions prevailing in the NBC as indicated by the clear response to starvation with respect to enhanced ammonium excretion.

The decrease in ammonium excretion during starvation, as it was observed in *E. hanseni* from the NBC, was also detected by Meyer and Oettl (2005); excretion rates decreased by 50 % in the first six days of starvation in larval *E. superba*. After this initial decline, however, excretion rates rose back to initial levels. Auerswald et al. (2009) reported that excretion rates of adult *E. superba* increased along the entire duration of the starvation experiment. In both studies the increase of ammonium excretion was explained as a consequence of protein breakdown during starvation. We neither saw an increase after 6 days of starvation nor an overall increasing trend in ammonium excretion through this study which complies with our findings that there was no change in total body protein over the time of starvation. However, a possible return to increasing excretion rates cannot be excluded as our experiments were only run for 7 days instead of 12 (Meyer and Oettl 2005) and 20 days (Auerswald et al. 2009).

Changes in the atomic O:N ratios corresponded to the species' oxygen consumption rates and ammonium excretion rates. In *E. hanseni* from the NBC, both rates decreased over the period of starvation, which resulted in constant O:N ratios with no statistical difference between the first and the last days of starvation in the 10°C and 15°C experiments. The ratios were consistently < 24 indicating a protein-dominated metabolism (Ikeda 1974). At 5°C, the respiration rates were not influenced by starvation which thus resulted in a slight increase in O:N ratios after the 7-day trials. *Euphausia hanseni* sampled in the ABF responded to the starvation stress with a decrease in respiration rates but showed no change in excretion rates, which therefore lead to a decline of O:N ratios over the time of starvation. This effect was primarily found in the 10 and 15°C treatments, where the initial ratios of ~ 30 (indicating a lipid-orientated metabolism) decreased over 7 days to ~ 10, corresponding with pure protein catabolism (Mayzaud and Conover, 1988).

The effect of short-term starvation indicated a negative relationship between the duration of food deprivation and CS activity as well as Michaelis-Menten constants (CS efficiency) between krill from either region. These results comply with Buchholz and Saborowski (Buchholz and Saborowski 2000, Saborowski and Buchholz 2002) in *M. norvegica* and support the idea that a reduction in the metabolic rate may be a compensatory effect of starvation; the specific activity of CS decreased (suggesting the reduction in metabolism) whereas the CS efficiency increased. Furthermore, the initial regional difference in Michaelis-Menten constants in *E. hanseni* remained significant over the time of starvation as animals from the NBC showed significantly lower enzyme efficiencies than specimens caught in the ABF. Considering Buchholz and Saborowski (Buchholz and Saborowski 2000, Saborowski and Buchholz 2002), the regional differences in enzyme efficiency support the assumption of unfavourable food conditions in the ABF and confirm the

suggestion that the animals from the ABF have already been under food deprivation before the experiment.

The analysis of elemental composition in terms of mean mass-specific C:N ratios revealed a slight decrease over the period of starvation in all specimens and at all temperatures. This suggests the preferential use of carbon containing energy reserves for the fulfilment of energy requirements during the initial period of food depletion in correspondence with findings on adult and larval Antarctic krill (Meyer and Oettl 2005, Auerswald et al. 2009). According to Auerswald et al. (2009), the proximate biochemical composition of Antarctic krill declined significantly with the starvation time with respect to the total lipid and total glycogen content in % DW^{-1}. However, significant decreases were not observed until day 15 of starvation. In our short-term experiments, no differences appeared over the time of starvation with regard to the total lipid and protein contents. This may be explained by an insufficient duration of the experiment and/or the fact that lipids, which are believed to be the main energy source in krill, were inherently very low and close to the critical minimum of 5 % DW^{-1} for survival (Hagen et al. 2001).

2.5 Conclusion and outlook

Euphausia hanseni adjusted its overall metabolic rates in response to a prevailing temperature gradient. Strong regional distinctions in ammonium excretion were found which related to the nutritional situation believed to prevail in each region. In the ABF unfavourable food conditions were observed first hand. Low phytoplankton concentrations may have resulted in a nutritional shift from a herbivorous to a carnivorous diet (as also indicated by O:N-ratios and stable isotope analyses). To confirm these results, further studies involving stomach content analyses, analysis of fatty acids as well as determination of digestive enzymes as trophic markers are envisaged.

A major focus of our study was to observe the starvation capability of krill from an upwelling system during late austral summer. *Euphausia hanseni* adapted quickly to food deprivation by down-regulating metabolic parameters helping to remain metabolically efficient at least over 7 days of starvation. This metabolic compensatory effect might also be viewed as an indicator for the adaptive capacity to overcome longer low-food periods between two consecutive upwelling events. Conversely, the substantial decrease of the metabolic parameters combined with the severely lower lipid content of ~ 8 % DW^{-1} may also be taken as an indication of a limited capability to overcome longer periods of starvation, i.e. longer periods of the absence of food. In fact, the experimental period was probably too short to confidently determine changes in structural physiological parameters such as elemental and proximate biochemical composition. Accordingly, in future experiments, longer periods would be helpful to verify the trends detected and to find the critical point in the starvation capacity. Furthermore, the “point-of-no-return” of adult *E. hanseni* may be defined by adding re-feeding experiments to the protocol [so far known from starvation experiments on crustacean larvae; e.g. Anger and Dawirs (1981), Ross and Quetin (1989)].

Krill’s physiological response to short-term starvation differed with regard to their region of origin (NBC/ABF). During the season investigated, specimens from the AC-influenced water masses (the ABF) reacted more dramatically, which supports the hypothesis that frequent short-term food deprivations lead to unfavourable trophic conditions in this tropical region.

Our study suggests a variety of methods that are applicable in other ecophysiological studies. For regional comparisons of nutritional condition, analyses of enzyme kinetics,

excretion rate measurements and stable isotope analyses are useful. The latter is easiest to perform to obtain first proximate information regarding differences in nutrition and foraging habitat. For a worldwide species comparison (Buchholz et al. 2010) and the determination of temperature affecting physiological performance, the measurements of the metabolic rates serve as a suitable descriptor for the overall metabolic activity and the resulting energy demand of the species. Furthermore, the ecophysiological data collected here might be useful for ecosystem modelling purposes and might serve as a baseline for further investigations on krill communities in upwelling systems. The comparison with other krill species will extend the applicability of krill as a biological indicator in ecosystem analysis.

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The high Arctic Kongsfjord (Storholmen Island in the centre) in April 2013

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3 PUBLICATION II

The other krill: overwintering physiology of adult *Thysanoessa inermis* (Euphausiacea) from the high Arctic Kongsfjord

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Abstract

Polar environments like the high Arctic Kongsfjord are determined by pronounced seasonality leading to strong variations in primary production. Particularly during winter, food sources are scarce. Herbivorous krill, like the arcto-boreal *Thysanoessa inermis* are key components in the ecosystem of Kongsfjord and strongly relies on phytoplankton as a food source. Therefore, during polar night such species must be adapted to survive long periods without significant nutritional input. We investigated physiological mechanisms and the allocation of energy resources to explain how *T. inermis* manages to survive the Arctic winter. Adult specimens were caught in late summer and kept under starvation conditions for 28 days. Changes in metabolic rates (respiration and excretion) and biochemical composition (protein, lipid and fatty acid analyses) were monitored. In contrast to the Antarctic krill, *Euphausia superba*, and the subtropical *E. hanseni*, the arcto-boreal species did not reduce metabolism but used lipid reserves for survival. Assessed from total lipid stores and energy demand, the potential survival period was estimated at 63 days without food uptake, which is not sufficient to survive the entire winter. Results were compared to specimens that overwintered in-situ and discussed in relation to other euphausiids. In conclusion, *T. inermis* is well adapted to survive the Arctic winter provided that alternative food sources are available, but has a different strategy to cope with starvation than krill species from other latitudes.

3.1 Introduction

The high Arctic Kongsfjord is located at 79°N at the West Coast of Spitsbergen. As a high latitude ecosystem, it is characterized by pronounced seasonal oscillations in light regime and, consequently, primary production (Hop et al. 2002). During polar night, perpetual darkness and partial sea ice coverage lead to complete suppression of pelagic autotrophic primary production (Hop et al. 2002). As a consequence, zooplankton species at lower trophic levels relying on phytoplankton as a food source, like herbivorous Euphausiids, are exposed to a long period of food deprivation.

Euphausiids (krill) are pivotal components of the pelagic macrozooplankton community throughout the World Oceans including the Arctic and subarctic marine ecosystems. They appear in substantial numbers and occupy a central trophic position by directly linking primary production to higher trophic levels with high efficiency (Falk-Petersen et al. 2000, Dalpadado and Mowbray 2013). The krill community of the Kongsfjord is primarily dominated by the arcto-boreal *Thysanoessa inermis* (Buchholz et al. 2010), an essentially herbivorous species. Due to its remarkable high lipid content mainly consisting of energy-rich wax esters (Sargent and Falk-Petersen 1981, Saether et al. 1986, Huenerlage et al. 2014), *T. inermis* plays a significant role in the energy transfer of the marine food web of the Kongsfjord. It is believed to be the main nutritional resource for many adult fish, seabirds and marine mammals

regularly occurring in the Kongsfjord (e.g. Dalpadado and Mowbray 2013, Lydersen 2014). Particularly over winter, when because of diapause calanoid copepods are rare in the water column (e.g. Auel et al. 2003), *T. inermis* provide a sustained and stable source of energy rich nutrition for planktivorous predators.

In Arctic waters, *T. inermis* has a life span of 3-4 years (Dalpadado and Skjoldal 1996). It must therefore have some specific adaptation to survive long periods of food absence during several Arctic winters. Deduced from its high lipid content, the species may contain enough energy to survive the winter without (or only a minimum) of food uptake (Falk-Petersen et al. 2000). In particular, fatty acid analyses indicated a temporary dietary shift to benthic detrivorous and carnivorous feeding (Sargent and Falk-Petersen 1981). As a further strategy, *T. inermis* could reduce its energetic costs by body shrinkage, sexual regression (Dalpadado and Ikeda 1989) and significant reduction of metabolic rates (e.g. Auerswald et al. 2009, Huenerlage and Buchholz 2013).

Nevertheless, to date it is not yet known from direct experiments how this key species reacts metabolically to food absence; i.e. how *T. inermis* regulates its overall metabolism in detail to meet energetic requirements during the Arctic polar night.

Starvation experiments are suited approaches to uncover the metabolic survival strategy of a species during times of food scarcity, i.e. over winter (Atkinson et al. 2002, Kreibich et al. 2008). In krill, such experiments were applied in order to reveal the overwintering strategy of Antarctic *Euphausia superba* (Ikeda and Dixon 1982, Auerswald et al. 2009) or reveal the survival strategy between upwelling pulses of adult *E. hanseni* from the Benguela upwelling (Huenerlage and Buchholz 2013).

Accordingly, in our present study we performed a starvation experiment which aimed at elucidating the specific survival pattern and metabolic performance of the arcto-boreal *T. inermis* during 28 consecutive days of food absence. To best predict the starvation induced metabolic changes we investigated a variety of physiological parameters throughout the experiment. Next to general life parameters (e.g. sex, size, weight, mortality, moulting), metabolic rates (respiration and ammonium excretion), lipid dynamics (total lipid, lipid class and fatty acid composition) as well as the elemental and biochemical composition (carbon, nitrogen and protein content) were monitored. Furthermore, based on energy demand and total lipid storage, we calculated the maximum potential duration of survival under starvation conditions.

To compare the findings from the starvation experiment, the same set of analyses was applied to specimens sampled in the early spring of the following year, i.e. as a reference for *T. inermis* having overwintered in the Kongsfjord in-situ. Our results on the adaptational starvation capacity will be compared to euphausiid species from other regions particularly to the Antarctic *E. superba* and the subtropical upwelling species *E. hanseni*.

3.2 Material and methods

3.2.1 Sample collection

Adult *Thysanoessa inermis* were sampled in the first week of August 2012 (August, 7th 2012; 78.97°N, 12.28°E) and in the first week of April 2013 (April, 6th 2013; 78.96°N, 11.94°E) on-board the Kings Bay AS workboat MS *Teisten* in the high Arctic Kongsfjord, W-Spitsbergen. The hauls were taken from 90 m (August 2012) and 300 m depth (April 2013) and operated at a speed of 2 knots using a 1 m^2 Tucker trawl (1000 µm mesh size and soft cod-end bucket). Shortly after catch, the specimens were transferred to aerated aquaria containing filtered seawater and given an acclimation period of 12 h at 4°C in dim light, before use in the experiments (respiration measurements and starvation experiment).

At the end of the experiments, specimens were scored for individual visible life parameters, namely: sex, size (from the front of the eyes to the tip of the telson to the nearest mm), fresh weight, stomach fullness, hepatopancreas colour, lipid stage (Buchholz et al. 2010), sexual development and moult stage (Buchholz 1982). Afterwards, the specimens were rinsed in distilled water, deep-frozen in liquid nitrogen and stored at -80°C for later analyses at the Alfred Wegener Institute, Bremerhaven (Germany).

3.2.2 Starvation experiments

A total of 96 adult *T. inermis* were chosen for the starvation experiment. To follow each specimen's condition over the time of starvation, i.e. as an estimation of start conditions, specific life parameters [stomach fullness, hepatopancreas colour and lipid stage (Buchholz et al. 2010)] were determined before the specimens were individually placed in 1 L Kautex bottles filled with filtered seawater of 4°C (provided by Kings Bay Marine Lab, Ny-Ålesund). The bottles were kept in a water bath at dim light. The experiment lasted 4 weeks (28 d). The specimens were checked twice daily for mortality and moulting and moulted carapaces were immediately removed from the bottles.

The intermoult period (IMP) was calculated according to Tarling et al. (2006). Only the moults occurring within the first 3 days were considered in order to reduce laboratory artefacts (Buchholz 2003).

Water exchange and subsampling were performed every 5th day and on the last (28th) day of starvation (T28). Subsamples were used for direct metabolic rate measurements (respiration and excretion) and later for analyses on biochemical composition (lipid compositions, protein content and carbon (C) to nitrogen (N) mass ratio).

Furthermore, a sample of 8 *T. inermis* specimens served as a control group (T0) for metabolic rates, lipid composition and overall specimen's condition at the start of the experiment.

3.2.3 Metabolic rate measurements

The respiration measurements were done on female and male *T. inermis* sampled at the beginning of the starvation experiment (T0) and on the 5th, 10th, 15th, 20th and 25th day of starvation (T5 - T25) as well as after overwintering (OW; specimens sampled in April 2013). The specimens were individually incubated in specially designed horizontal tubular chambers [Perspex; total volume of 20 ml; Huenerlage and Buchholz (2013)]. The chambers were filled with filtered seawater of 4°C and stored in a water bath in the dark in a temperature controlled refrigerator. Two chambers were prepared without an animal to serve as controls. The oxygen consumption (mg O_2 L^{-1}) was monitored every 30 seconds by optode respirometry

with a 10-channel optode respirometer (PreSens Precision Sensing Oxy-10 Mini, Regensburg, Germany). The measurements lasted about 3 h and were stopped when the oxygen concentration inside the test-chambers reached 60 % of the start concentration.

Ammonium (NH_4-N) excretion was determined from a water sample (500 µl) taken at the end of each respiration measurement. The samples were frozen and stored at -80°C until measurement. The analysis was done photometrically using a microplate reader (630 nm; Multiskan™ FC Microplate Photometer, Thermo Scientific, USA) following the phenol-hypochlorite method according to Solorzano (1969).

Both the respiration rates and the ammonium excretion rates were expressed in µmol h^{-1} and referred to gram fresh weight (FW).

Derived from the metabolic rate measurements, the oxygen (O) to nitrogen (N) ratio was used as an indication for the substrate metabolized: O:N < 24 for protein and O:N > 24 for lipid (Mayzaud and Conover 1988).

3.2.4 Biochemical composition

The biochemical composition was analysed on female *T. inermis* sampled on the 5th and the 25th day of starvation (T5 and T25). Whole animals were lyophilized for 24 h, their dry weights determined and ground to powder using a glass tissue grinder.

Total protein was determined using the Bio-Rad *DC* Protein Assay (Bio-Rad, Munich, Germany). 2 to 3 mg of krill powder were homogenated in 1 mL NaOH (1M) by using an ultrasound sonotrode at about 35 % of maximum power (BRANSON SONIFIER® cell disruptor B15, Germany) and incubated for 2 h at 60°C. Afterwards, the homogenate was centrifuged at room temperature for 10 min at 10 000 g. The supernatant was taken for photometrical determination using a microplate reader (750 nm; Multiskan™ FC Microplate Photometer, Thermo Scientific, USA). The measurements were performed in triplicates. Bovine albumin serum served as standard.

Analyses on the carbon and nitrogen content used the same amounts of the krill powder and were performed in an elemental analyser (Euro EA CHNS-O elemental analyser, HEKAtech GmbH, Germany). Acetanilide was used as standard.

3.2.5 Lipid analyses

The lipid composition was analysed on female specimens sampled at the beginning of the starvation experiment (T0), on the 15th and 28th day of starvation (T15 and T28) as well as after overwintering (OW; specimens sampled in April 2013).

Lipids were extracted from lyophilized whole specimens (24 h) of known dry weight. The total content was determined gravimetrically (Hagen 2000) and expressed as percent of dry weight. The extractions were performed in the laboratories of the department of Marine Zoology, University of Bremen (BreMarE, Germany).

The lipid classes were analysed from aliquots of the total lipid extracts. The separation and identification was done on a silica column (Chromolith®Performance-Si 100 x 4.6 mm i.d.) using high performance liquid chromatography (LaChromElite HPLC system; VWR, Darmstadt, Germany) with an SEDEX 40 evaporative light scattering detector (Graeve and Janssen 2009). A commercially available standard representing a single lipid class was used for identification. Lipid classes were quantified as percent of total lipid.

Fatty acid and fatty alcohol compositions were identified according to Kattner and Fricke (1986). Aliquots of the total lipid extracts were hydrolysed in methanol containing 3 % concentrated sulphuric acid under nitrogen atmosphere and transesterificated for 4 h at 80°C. Subsequent analyses were done by gas liquid chromatography (HP 6890N GC) on a wall-coated open tubular column (30 x 0.25 mm inside diameter; film thickness: 0.25 µm; liquid phase: DB-FFAP) using temperature programming. Standard mixtures served to identify the fatty acid methyl esters and the fatty alcohol derivates. If necessary, further identification was done by GC-mass spectrometry using a comparable capillary column. Detailed fatty acid and alcohol composition were expressed as percent of total fatty acid and percent of total fatty alcohols, respectively.

3.2.6 Calculation of potential maximum starvation

The potential maximum starvation period of *T. inermis* was estimated from the available mean energy deposit and the mean daily energy requirements found in unstarved specimens at the start of the starvation experiment. From this, the theoretical loss in lipid over the time of the experiment was estimated.

For the calculation of the energy deposit of an average specimen, the mean available lipid deposit (mg) was converted to energy units (J) assuming a caloric equivalent of 42.69 J mg^{-1} for wax ester dominated lipid (Auel et al. 2003).

The average daily energy requirement was calculated from the mean individual`s respiration rate (µmol O_2 d^{-1} $individual^{-1}$) assuming a caloric equivalent of 0.44 J per µmol of oxygen consumed for lipid (Ikeda et al. 2000).

3.2.7 Statistics

The statistical analyses were performed using the GraphPad Prism 5 software (GraphPad Software, Inc., USA). A one-way ANOVA with post-hoc Dunnett test was used to detect significant differences between starved (T5-T28) and non-starved (T0) krill. Differences between the specimens overwintered (OW) and the field samples of August 2012 (T0) were analysed with an unpaired t-test. The same test was used to compare OW specimens to *T. inermis* from the starvation experiment (T28). The significance level was set at $p < 0.05$. All results are given as means ± standard deviations unless specified otherwise.

3.3 Results

The starvation experiment used a total of 104 specimens and was composed of 50 females, 47 males (both sexually inactive; i.e. neither ovaries nor spermatophores were visible) and 7 neuters. In the latter, the degree of sexual regression did not allow for sex determination. At the beginning of the experiment, the majority of the specimens had empty stomachs (80 %) and colourless hepatopancreases (87 %). The mean lipid stage at the start was 4.2 ± 0.5, i.e. a high lipid store. The specimens were on average 24.7 ± 1.8 mm long and had a fresh weight of 119.1 ± 25.8 mg (30.0 ± 1.6 mg dry weight, Table 3.1). The survival rate during the 28 days was 80 %. The in-situ intermoult period of the specimens sampled in August 2012 was ~ 11 days. Continuous investigations on the individuals' moult stages showed that the specimens remained actively moulting throughout the experiment maintaining the initial intermoult period.

The overwintered specimens (OW) sampled in April 2013 were still sexually inactive (sexual development stage = 0). All of them had empty stomachs and colourless hepatopancreases. The mean lipid stage was 3.6 ± 0.7 and significantly lower compared to the specimens from

August 2012 ($p < 0.01$, t = 2.7, df = 109). Additionally, although of about the same body length (23.5 ± 1.7 mm), the specimens from April 2013 had a lower fresh weight of 91.0 ± 26.5 mg ($p < 0.05$, t = 2.6, df = 26; 22.7 ± 6.7 mg dry weight, Table 3.1).

Table 3.1 *Elemental and biochemical composition of female Thysanoessa inermis during starvation (T0 – T28) and after overwintering (OW; specimens caught in April 2013). Values are given as means ± SD. DW = dry weight; C = body carbon; N = body nitrogen; n = number of individuals.*

	Days of starvation					
	T0 (*n* = 4)	T5 (*n* = 5)	T15 (*n* = 4)	T25 (*n* = 5)	T28 (*n* = 4)	OW (*n* = 4)
Dry weight (mg)	29.3 ± 5.7	30.9 ± 6.7	32.6 ± 4.1	25.1 ± 1.6	20.9 ± 1.4	22.7 ± 6.7
Total lipid (% DW)	42.3 ± 1.1	-	41.2 ± 7.6	-	27.6 ± 6.6	32.2 ± 2.4
Total protein (% DW)	-	34.2 ± 2.3	-	36.3 ± 2.8	-	-
C:N mass ratio	-	6.6 ± 0.8	-	6.2 ± 0.6	-	-

3.3.1 Metabolic rates

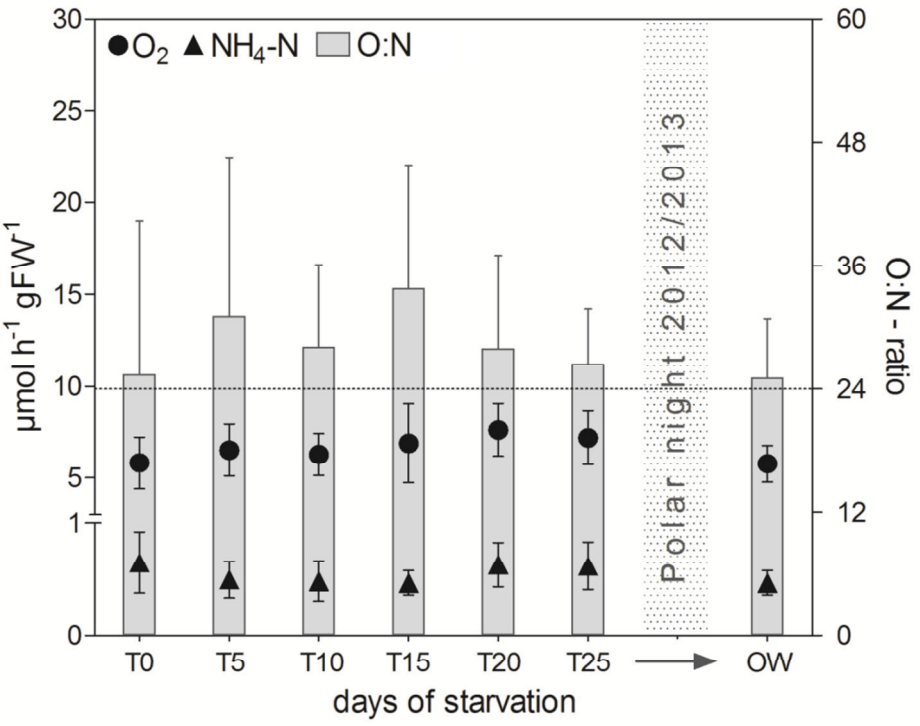

Fig. 3.1 *Oxygen consumption (O_2), ammonium excretion (NH_4-N) and atomic oxygen to nitrogen ratio (O:N) at 4°C of adult Thysanoessa inermis (n = 8) over time of starvation (T0 – T28) and after overwintering (OW; specimens from April 2013). Values are given as means ± standard deviations. Dotted line at O:N-ratio = 24 indicates equal use of proteins and lipids (Mayzaud and Conover 1988). FW = fresh weight.*

Both the respiration rates and the excretion rates did not change over time of starvation (T0 – T28) and were the same in specimens sampled in early April 2013 (OW). Accordingly, the atomic oxygen to nitrogen ratio (O:N) was not different between all specimens sampled (Fig. 3.1). At 4°C experimental temperature, mean respiration rates ranged on average from ~ 6 µmol O_2 h^{-1} gFW^{-1} (at days of starvation T0 – T10 and OW; Fig. 3.1) to ~ 7 µmol O_2 h^{-1} gFW^{-1} (at days of starvation T15 – T25). Excretion rates ranged on average from ~ 0.5 µmol NH_4-N h^{-1} gFW^{-1} (at days of starvation T5-T15 and OW) to ~ 0.6 µmol NH_4-N h^{-1} gFW^{-1} (at days of starvation T0, T20 and T25). Mean O:N-ratios were > 24 in all treatments suggesting preferential catabolism of lipids (Fig. 3.1).

3.3.2 Elemental and biochemical composition

On the last day of the starvation experiment, the specimens had a significantly lower dry mass (20.9 ± 1.4 mg) compared to specimens from the beginning (30.0 ± 1.6 mg; $p < 0.01$, F = 4.5; Table 3.1). However, total body protein (~ 35 %) and carbon to nitrogen mass ratio (C:N; ~ 6) remained stable over the time of starvation (Table 3.1). In contrast, total lipid decreased considerably from ~ 42 % at the beginning (T0) to ~ 28 % at the end of the experiment (T28; $p < 0.05$, F = 4.5; Table 3.1). Simultaneously, the size of the lipid body was visibly decreasing ($p < 0.05$, F = 2.9; Fig. 3.2).

Mean relative lipid class composition was changing during the experiment (Fig. 3.3). The relative amounts of both the triacylglycerols (from ~ 17 % at T0 to 14 % at T28) and wax esters (from ~ 50 % at T0 to 44 % at T28) were decreasing whereas those of polar lipids (PC: from ~ 29 % at T0 to ~ 35 % at T28; PE: from ~ 3 % at T0 to 5 % at T28) were increasing. Due to high variance, the differences were not significant. The increase in sterols (from ~ 1 % at T0 to 3 % at T28), however, was statistically different ($p < 0.05$, F = 5.6).

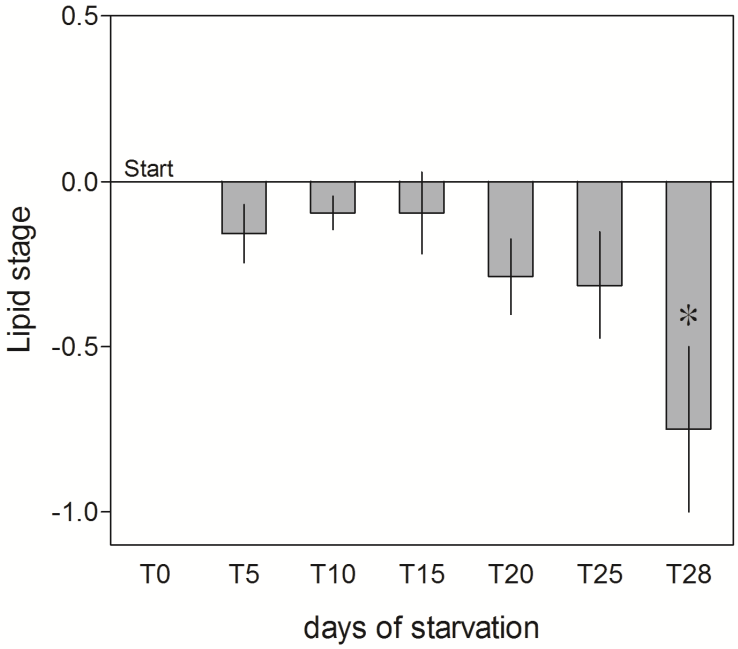

Fig. 3.2 *Mean change in the lipid stages of Thysanoessa inermis (n = 8 – 16) over time of starvation (T0 – T28). Vertical lines represent the standard errors of the means. * Significant difference to T0.*

In all specimens sampled, the total lipids consisted of ~ 17 % fatty alcohols and ~ 83 % fatty acids. 5 fatty alcohols and 13 fatty acids were detected accounting for > 1 % to the total composition (Table 3.2). 14:0, 16:0 and 16:1(n-7) were dominating the fatty alcohol composition (~ 25 %, ~ 60 % and ~ 16 % of total fatty alcohols respectively). The relative amounts of these alcohols did not change during starvation (Table 3.2). Both 20:1 and 22:1 fatty alcohols were present in traces in *T. inermis* sampled at the beginning of the experiment but were absent after starvation (T28).

Table 3.2 *Fatty acid and alcohol compositions (% of total fatty acids and alcohols) of female Thysanoessa inermis during starvation (T0 – T28) and after overwintering (OW; specimens caught in April 2013). Values are given as mean percentages ± standard deviations. Compounds < 1 % are not shown or marked as traces (tr) as soon as they were present in comparative specimens. SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids; n = number of individuals.*

	Days of starvation			
	T0	T15	T28	OW
Composition	(*n* = 4)	(*n* = 4)	(*n* = 4)	(*n* = 4)
Fatty acid				
14:0	2.4 ± 0.1	2.5 ± 0.5	2.3 ± 0.3	2.9 ± 0.6
16:0	21.7 ± 0.9	23.0 ± 1.0	20.8 ± 1.5	20.8 ± 1.0
18:0	1.8 ± 0.4	2.0 ± 0.1	2.0 ± 0.2	1.6 ± 0.4
Σ SFAs	25.9 ± 1.2	27.5 ± 1.4	25.0 ± 1.9	25.3 ± 0.9
16:1(n-7)	12.7 ± 1.5	11.6 ± 4.1	11.2 ± 1.4	6.9 ± 4.1
18:1(n-9)	22.3 ± 0.7	22.7 ± 1.1	22.0 ± 2.3	22.3 ± 3.4
18:1(n-7)	12.2 ± 1.0	10.1 ± 0.8	11.2 ± 1.5	9.3 ± 1.8
20:1(n-9)	tr	tr	tr	2.9 ± 3.9
22:1(n-11)	tr	tr	tr	1.3 ± 2.3
Σ MUFAs	49.0 ± 1.6	46.1 ± 3.8	46.2 ± 3.6	43.6 ± 4.1
16:2(n-4)	1.2 ± 0.2	1.0 ± 0.3	1.1 ± 0.1	tr
18:2(n-6)	tr	1.1 ± 0.6	1.1 ± 0.1	2.4 ± 0.6
18:4(n-3)	2.2 ± 0.1	3.1 ± 1.6	2.0 ± 0.3	4.8 ± 2.6
20:5(n-3)	15.3 ± 1.0	15.3 ± 1.3	15.6 ± 0.7	14.4 ± 0.6
22:6(n-3)	4.5 ± 1.4	4.6 ± 1.3	7.3 ± 1.2	6.9 ± 1.3
Σ PUFAs	25.1 ± 1.1	26.3 ± 2.4	28.8 ± 1.9	31.1 ± 3.7
Fatty alcohol				
14:0	24.7 ± 2.1	25.7 ± 4.6	24.8 ± 0.5	29.9 ± 4.1
16:0	57.6 ± 2.4	58.2 ± 1.5	57.5 ± 1.6	51.8 ± 10.7
16:1(n-7)*	16.2 ± 2.2	15.6 ± 4.8	17.8 ± 1.6	9.6 ± 4.4
20:1(n-9/n-7)	tr	tr	-	4.0 ± 7.0
22:1(n-11/n-9)	tr	-	-	4.7 ± 8.8

*might coincide with small amounts of 16:4(n-1) fatty acid

Within the fatty acids, 16:0, 16:1(n-7), 18:1(n-9), 18:1(n-7) and 20:5(n-3) were detected in highest amounts in all specimens. Long-chain 20:1(n-9) and 22:1(n-11) fatty acids were only found in traces (< 1 %). Except for the increase in the amount of 22:6(n-3) fatty acid ($p < 0.05$, F = 4.6; Table 3.2), there was no statistical difference in the fatty acid composition of *T. inermis* over time of starvation. However, mean values of 16:0 and 16:1(n-7) showed a decreasing trend whereas those of 18:2(n-6) and 22:6(n-3) were slightly increasing.

In-situ overwintered *T. inermis* (OW) sampled in April 2013 were statistically different compared to unstarved (T0) specimens from August 2012. The dry weight was significantly less (~ 23 mg; $p < 0.05$, t = 2.9, df = 8) and the total lipid content was 10 % lower (~ 32 % of dry weight; $p < 0.001$, t = 7.6, df = 6; Table 3.1). Within the lipid classes, sterols were present in higher amounts (~ 2 % of total lipid; $p < 0.05$, t = 2.8, df = 6; Fig. 3.3). The fatty acid composition was dominated by the same fatty acids like in the specimens of August 2012. Nevertheless, 18:2(n-6) was present in higher amounts ($p < 0.01$, t = 5.1, df = 6) whereas 16:1(n-7), 18:1(n-7) and 16:2(n-4) were found in lower amounts ($p < 0.05$, t = 2.6, df = 6; $p < 0.05$, t = 2.7, df = 6; $p < 0.01$, t = 3.7, df = 6). Furthermore, the 18:4(n-3) fatty acid and the long-chain 20:1 and 22:1 fatty acids and alcohols were found in higher amounts although not statistically significant due to high standard deviations (Table 3.2). Within the fatty alcohols, however, 16:1(n-7) was present in lower amounts in *T. inermis* from April 2013 compared to the specimens from August 2012 ($p < 0.05$, t = 2.7, df = 6).

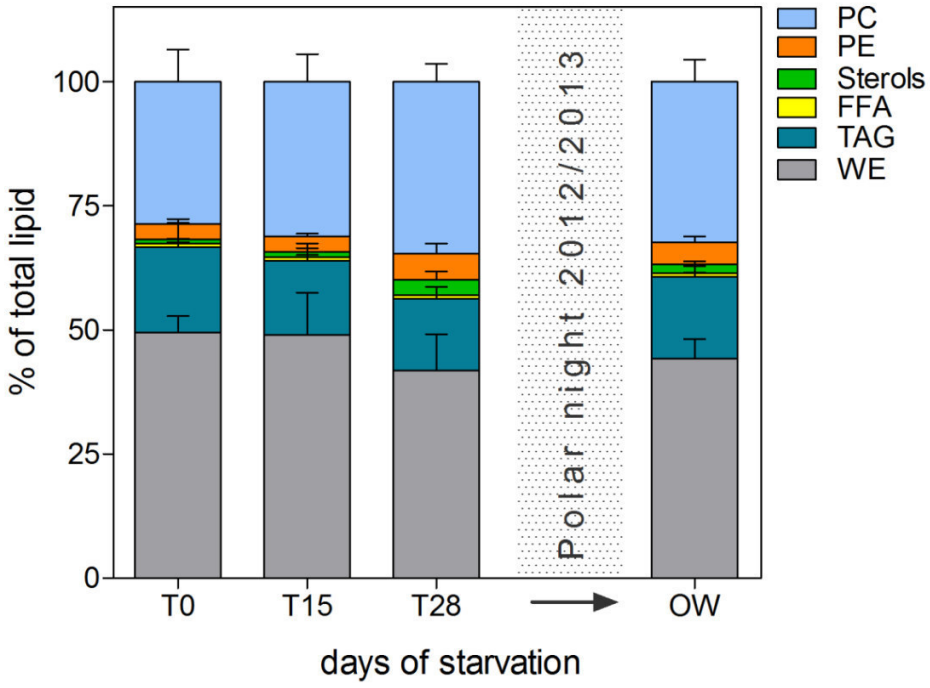

Fig. 3.3 *Lipid class composition of female Thysanoessa inermis (n = 4) over time of starvation (T0 – T28) and after overwintering (OW; specimens from April 2013). Percentages are shown as means ± standard deviations. WE = Wax ester; TAG = Triacylglycerol; FFA = Free fatty acids; PE = Phosphatidylethanolamine; PC = Phosphatidylcholine.*

In relation to starved individuals of *T. inermis* (T28), in-situ overwintered specimens were not different with regard to dry weight, total lipid and lipid class composition (Table 3.1; Fig. 3.2).

Within the fatty acids, the difference was only significant in 16:2(n-4) (traces in OW compared to ~ 1.1 % at T28; $p < 0.05$, t = 2.9, df = 5) and 18:2(n-6) (~ 2.4 in OW compared to traces in T28; $p < 0.05$, t = 3.6, df = 5 respectively; Table 3.2).

3.3.3 Potential maximum starvation

The daily individual energy demand was 7.6 J (~ 0.17 mg lipid) calculated from the mean respiration rate of 6 µmol O_2 h^{-1} gFW^{-1} (Fig. 3.1) and a fresh weight of ~ 120 mg. Assuming an essential minimum lipid content of 5 %, individual *T. inermis* contained ~ 11.2 mg of potential consumable lipid (37.3 % of ~ 30 mg dry weight; Table 3.1), which is equivalent to an energy storage of ~ 477.7 J available for overwintering. Accordingly, the lipid reserves alone could cover the energy requirements of adult *T. inermis* for ~ 63 days.

3.4 Discussion

The overwintering experiment was performed on adult *Thysanoessa inermis* at the end of the Arctic summer. During this time, phytoplankton, i.e. chlorophyll *a* concentration, was already low (<0.5 µg l^{-1}; ref. COSYNA data web portal 'Underwaternode Spitsbergen' operated by AWI and the Helmholtz-Zentrum Geesthacht Zentrum für Material- und Küstenforschung GmbH; http://codm.hzg.de/codm) compared to spring bloom situations, in which the concentration can reach 13 µg l^{-1} (Hegseth and Tverberg 2013). This was also reflected in the nutritional condition of *T. inermis* at the start of the experiment. Generally, during times of high food supply, the colour of the midgut gland depends on the specific source of nutrition. Unlike that, most of the specimens had an empty stomach and colourless hepatopancreas indicating only occasional or even no feeding before catch.

Nevertheless, high lipid contents (~ 42 %) in the specimens revealed that *T. inermis* was well prepared for the upcoming winter. To avoid sex specific influences (e.g. Clarke 1980), only female specimens were used for lipid analyses. However, under the condition that the specimens were sexually regressed, Huenerlage et al. (2014) found no statistical differences in the lipid composition of adult male and female *T. inermis*.

Sexual regression was also found in our study. All of the specimens sampled for the experiment in August 2012 were sexually inactive. In ~ 7 % of the specimens, the sexual regression was progressed to such an extent, that a clear sex determination was not possible. In euphausiids, sexual regression is a common functional response to longer periods of food scarcity. It is an adaptation to reduce metabolic costs when species are unable to sufficiently cover reproductive needs, e.g. during overwintering, as found in the Antarctic *Euphausia superba* (Kawaguchi et al. 2007), the boreal *Meganyctiphanes norvegica* (Cuzin-Roudy and Buchholz 1999) but also in *T. inermis* from the Barents Sea (Dalpadado and Ikeda 1989). Hence, our study confirmed, that *T. inermis* may take advantage of this adaptation to save energy especially over winter. As the regression had already started in late summer, it likely was triggered by the already limited food supply (Dalpadado and Ikeda 1989), indicated by the low chlorophyll *a* concentration in the first week of August 2012 (<0.5 µg l^{-1}; see above).

Body shrinkage is a further common morphological adaptation induced by inadequate nutrition (e.g. Meyer 2012 and references therein) being used like sexual regression, to reduce the loss of metabolic energy during times of food scarcity. Shrinkage was first described for experiments on the overwintering mechanisms of the Antarctic *E. superba* (Ikeda and Dixon 1982), where negative growth despite continuous moulting was found. In our experiment, the intermoult period remained stable and was defined at ~ 11 days at 4°C

corresponding to values in previous studies on *T. inermis* at 5°C (Dalpadado and Ikeda 1989). However, *T. inermis* which had overwintered in the field and was sampled in the first week of April 2013, showed the same mean body length as the specimens sampled in August 2012. Like in *E. superba*, *T. inermis* shows negative growth over winter, i.e. from August to February the following year (Dalpadado and Skjoldal 1996). Hence, as positive growth likely re-started in February, the specimens sampled in April 2013 may have already compensated body shrinkage and therefore exhibited the same size as before winter.

One of the most pronounced responses to starvation in euphausiids and other crustacean zooplankton (e.g. copepods) is a significant decrease in respiration rate (e.g. Quetin and Ross 1991, Auel et al. 2003, Meyer 2012). Euphausiids from other latitudes, like the adult Antarctic *E. superba* and the southern Atlantic upwelling species *E. hanseni*, down regulated their respiration rates by up to 70 % during winter and/or food absence respectively (e.g. Meyer 2012 and references therein, Huenerlage and Buchholz 2013). In *T. inermis,* however, the respiration rate did not change with time of starvation and was the same in the overwintered specimens sampled in April 2013. It is an exception, from the respiration point of view, that metabolic costs remained stable in *T. inermis*. It neither reduced its overall metabolism during the starvation experiment nor was it found to have done so right after winter. Instead, the mean respiration rate (~ 6 µmol O_2 h^{-1} gFW^{-1}) stayed comparable to unstarved euphausiids investigated at in-situ temperatures from other latitudes (e.g. Ikeda and Mitchell 1982, Saborowski et al. 2002, Auerswald et al. 2009, Huenerlage and Buchholz 2013).

Like the respiration rates, excretion rates did not change. Both, the specimens subjected to the starvation experiment and overwintered, krill had excretion rates ~ 0.55 µmol NH_4-N h^{-1} gFW^{-1}. Excretion is closely related to the food ingested (e.g. Saborowski et al. 2002). Hence, high phytoplankton concentrations correlate with high ammonium excretion rates, whereas the absence of food leads to a strong decrease in excretion within days (e.g. Huenerlage and Buchholz 2013). In August 2012, *T. inermis* had already been exposed to food depletion before catch (see above). Accordingly, the excretion rate was most likely already at its minimum at the start of the experiment and therefore did not change any more.

In all *T. inermis* sampled, the atomic oxygen (O) to nitrogen ratio (N) pointed to a lipid dominated metabolism (Mayzaud and Conover 1988). The mean O:N-ratio stayed above 24, both in the specimens from August 2012, over the time of starvation and after in-situ overwintering in April 2013. Concomitantly, lipid analyses showed a clear decrease in total lipid over the time of starvation, whereas the protein content remained stable. In euphausiids, proteins are reported to play a minor role in the allocation of energy and are known to be only metabolized after the depletion of lipid stores (Torres et al. 1994, Auerswald et al. 2009). Hence, our results showed that under food depletion the metabolic energy demand of *T. inermis* is mainly covered by the catabolism of storage lipids.

In most krill species, lipids are stored in triacylglycerols (Saether 1986). In contrast, *T. inermis* primarily uses wax esters (~ 50 % of total lipid in the specimens of this study) which are specifically biosynthesized during the exploitation of the spring and (early) summer phytoplankton blooms (Falk-Petersen et al. 2000). Compared to triacylglycerols, wax esters have a higher calorific content and are considered as more persistent long-term energy depots (Lee et al. 2006). They are reported as a major adaptation in polar zooplankton but are commonly found in polar copepods (Scott et al. 2000). In euphausiids, this lipid class is only present in some species, e.g. in the Antarctic *Euphausia crystallorophias* and *Thysanoessa macrura* and in the north Atlantic *T. inermis* and its congeners *T. raschii* and *T. longicaudata* (e.g. Falk-Petersen et al. 2000, Huenerlage et al. 2014).

T. inermis uses both wax esters and triacylglycerols for energy provision (Saether et al. 1986). However, despite the significant decrease of total lipid over time of starvation and after overwintering, we did not find a significant change in any of the single lipid classes. Rather, wax esters and triacylglycerols showed a decreasing trend whereas the proportion of polar lipids increased. Additionally, although not of storage lipid function, the relative proportion of sterols was significantly increased, indicating a simultaneous catabolism of both the wax esters and the triacylglycerols. The total share of the polar lipids and the sterols did not change. This would explain the relative increase of these lipid classes in proportion of total lipids.

Based on the wax ester dominated lipid metabolism of *T. inermis*, we calculated a daily energy demand of ~ 7.6 J d^{-1}. Taking into account the mean metabolic costs of an individual and the critical lipid limit of 5 % essential for survival (Saether et al. 1986, Hagen et al. 2001), we assessed the potential survival time of adult *T. inermis* at 63 days. Hence, assuming a minimum starvation period of 116 days during polar night (Hop et al. 2002), i.e. irrespective of the already variable phytoplankton concentration in late summer (Hop et al. 2002, Eilertsen et al. 2009), the lipid depot alone would not be sufficient to cover the metabolic energy requirements of this species.

As a consequence, *T. inermis* must (at least occasionally) switch to alternative food sources to survive the winter. This assumption is further confirmed by the still high lipid contents found in the overwintered specimens that were sampled in pre-bloom conditions in the first week of April 2013 (Chlorophyll *a* concentration < 0.06 µg l^{-1}; source: COSYNA – see above). The total lipid of the specimens overwintered was even slightly higher compared to starved specimens at the end of the experiment in August 2012.

The analysis of fatty acid markers is frequently used to determine or confirm trophic relationships (Dalsgaard et al. 2003). Compared to traditional stomach analyses, which only reflect recent feeding, the determination of fatty acids is especially used to detect long-term nutritional changes (Dalsgaard et al. 2003 and references therein). In our study, fatty acids did not change over the time of starvation but indicated differences between the two seasons, i.e. at the start of the experiment in August 2012 and after over wintering in April 2013. The analysis of fatty acids was therefore useful to identify the potential prey composition for krill over winter.

The probable change to different food sources was mainly indicated by the significant decrease in diatom markers [16:1(n-7) and 18:1(n-7) (e.g. Stübing and Hagen 2009)] and the simultaneous increase in *Calanus* markers, i.e. long-chain 20:1(n-9/n-7) and 22:1(n-11/n-9) fatty acids and alcohols (e.g. Dalsgaard et al. 2003). However, with regard to the *Calanus* markers, high standard deviations indicated strong differences between the individuals investigated, indicating unpredictable food sources. Nevertheless, this finding serves as evidence of increased carnivory over winter. Calanoid copepods from the Arctic (i.e. *Calanus finmarchicus*, *C. hyperboreus* and *C. glacialis*) are characterized by high lipid contents depending on species, size and development stage (e.g. Scott et al. 2000, Auel et al. 2003, Falk-Petersen et al. 2009). Their lipid storage can even exceed 70 % of their dry weight (e.g. Falk-Petersen et al. 2009). Accordingly, *T. inermis* could obtain enough energy from only occasional feeding on these Arctic copepods. For example, the total digestion of one calanoid copepod *C. hyperboreus* (~ 55 J $individual^{-1}$; Auel et al. 2003) would supply enough energy to cover the energetic requirements of *T. inermis* for ~ 7 days. Additionally, *T. inermis* may follow the behaviour of its congener *T. raschii* and switch to temporary detritus and benthic feeding (Sargent and Falk-Petersen 1981).

In summary, we suggest the overwintering pattern of *T. inermis* as an effective combination of four different physiological characteristics: a) sexual regression, b) body shrinkage, c) use of internal lipid stores and d) occasional predation on calanoid copepods and potentially benthic feeding.

3.4.1 The other krill - interspecies comparison of overwintering patterns

In crustaceans, there are at least three strategies to survive periods of food scarcity, i.e. overwintering. They are defined through different energetic adaptations (Torres et al. 1994, Hagen 1999). According to Torres et al. (1994), euphausiids are classified to exhibit a 'Type 2' strategy. This strategy is, next to the use of internal body reserves and opportunistic feeding behaviour, basically characterized by a pronounced reduction in metabolic rates.

Metabolic reduction, as a result of food absence, was found in krill of different climatic zones like in North Atlantic *M. norvegica*, the subtropical *E. hanseni* and the Antarctic *E. superba* (e.g. Quetin and Ross 1991, Saborowski et al. 2002, Meyer et al. 2012, Huenerlage and Buchholz 2013). During overwintering in *E. superba*, for example, more than 71 % of the energetic costs are covered solely by lowering the metabolic rates (Quetin and Ross 1991). *T. inermis* in our study, however, did not at all reduce its overall metabolism during the time of starvation.

Furthermore, the mode of energy storage distinguishes this species from the other investigated krill species. Unlike *M. norvegica* and *E. superba* (both exhibiting triacylglycerols as energy depot), *T. inermis* shows higher amounts of total lipids and is able to *de novo* biosynthesize energy-rich wax esters as major energy storage (Falk-Petersen et al. 2000).

The difference is even more pronounced compared to the subtropical upwelling species *E. hanseni* which holds almost no storage lipids and is therefore not adapted to any longer period of starvation (Huenerlage and Buchholz 2013). In the highly productive upwelling system of the Northern Benguela Current, the species is consistently surrounded by a 'basic food supply', additionally supplemented by frequent short-term phytoplankton blooms, i.e. upwelling events (Werner and Buchholz, in prep.). Accordingly, *E. hanseni* is hardly exposed to extreme food limitation and is therefore not at all adapted to store lipids.

In contrast, its true polar Antarctic congener, *E. superba*, is well adapted to seasonally changing food availability, i.e. to long periods of food absence during austral winter (Meyer et al. 2012 and references therein, Schmidt et al. 2014). Nevertheless, during austral summer, it experiences a long period of food abundance as the Southern ocean is believed to be one of the most productive areas albeit with patchy distribution of food sources (Smith et al. 1998, Schmidt et al. 2014). However, *E. superba*'s pronounced metabolic compensation of food absence developed most likely under the pressure to survive the annual overwintering period.

For comparison, the arcto-boreal *T. inermis* experiences successive periods of low phytoplankton concentrations; in addition to the long period of food deprivation during polar night. In the Kongsfjord ecosystem, summer primary production underlies strong variations. These are partly determined by the decrease of the euphotic zone which is attributed to the seasonal increase in turbidity due to river runoff and glacial outflow (Eilertsen et al. 1989, Hop et al. 2002). Hence, in the high Arctic Kongsfjord, the only predictable phytoplankton production is occurring in late spring (Hop et al. 2002), when *T. inermis* accumulates its energy-rich wax ester storage lipids (Falk-Petersen et al. 2000).

According to Torres et al. (1994), wax ester storage is the major characteristic of the 'Type 1' survival strategy and generally exhibited by diapausing copepods. However, *T. inermis* does

not diapause, i.e. it remains active over winter and switches to alternative food sources. In turn, this is the common pattern in crustaceans exhibiting the 'Type 3' survival strategy; e.g. decapods, mysids and gammarid amphipods (Torres et al. 1994, Hagen 1999).

In summary, *T. inermis* shows a different overwintering strategy (i.e. metabolic response to starvation) compared to krill species from other regions. The survival pattern of *T. inermis* may be categorized as an intermediate between the 'Type 1' (linked by its high wax ester content) and the 'Type 3' strategy (linked by its constant metabolic activity and opportunistic feeding behaviour).

3.5 Conclusion

The arcto-boreal *T. inermis* appears to successfully cope with both successive and long periods of food limitation determined by strong seasonality. This is in contrast to the subtropical upwelling krill species *E. hanseni*, which follows a 'hand-to-mouth' existence with only limited capability to survive long-term food deprivation due to very low lipid reserves (Huenerlage and Buchholz 2013).

In most respects, the survival strategy of *Thysanoessa inermis* was found to be similar to the Antarctic *E. superba* and the Northern krill *Meganyctiphanes norvegica* (i.e. body shrinkage, sexual regression, use of internal energy storage and opportunistic feeding). However, differences were found in terms of the energy storage pattern (long-term wax ester storage in *T. inermis* vs. short-term triacylglycerols) and the outstanding characteristic of *T. inermis*, not to reduce its overall metabolism.

Accordingly, our study highlights the diversity in physiological responses to starvation of different krill species adapted to their different habitats. In future experiments, investigations on the species metabolic and digestive enzyme activities and kinetics may be useful to increase the knowledge on the physiological performance of *T. inermis* during winter, i.e. under starvation conditions (e.g. Kreibich et al. 2009, Freese et al. 2012).

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Arctic tern (*Sterna paradisaea*) feeding its chick with krill (*Thysanoessa* ssp.)

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4 PUBLICATION III

Lipid composition and trophic relationships of krill species in a high Arctic fjord

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Abstract

Our study deals with the lipid biochemistry of the krill community in the ecosystem of the high Arctic Kongsfjord (Svalbard). During the last decades, Kongsfjord experienced a change in krill species composition due to recent increased advection of Atlantic water masses carrying characteristic boreal as well as subtropical-boreal euphausiids into the ecosystem. The lipid biochemistry and trophic relationships of the species recently inhabiting the Arctic water masses are scarcely known, although a change in a krill population may have a significant impact on the ecosystem. A comparison of nutrition and energy storage strategies, stable isotopes, lipid profiles and fatty acid compositions showed remarkable differences between the krill species. These reflected the diverse feeding behaviours and specific adaptations to the environments of their origin: the boreal *Meganyctiphanes norvegica* and subtropical *Nematoscelis megalops* appear more carnivorous, have significantly lower mean lipid contents (29 % and 10 %, respectively) and a different energy storage pattern (triacylglycerols and polar lipids, respectively) than the arcto-boreal *Thysanoessa inermis,* which consists of up to 54 % of lipids mainly stored as wax esters (> 40 %). These differences may have significant implications for the rapidly changing marine food-web of Kongsfjord - especially for higher trophic levels relying on the nutritional input of animal lipids.

4.1 Introduction

The high Arctic Kongsfjord is located at the West Coast of Svalbard (79°N). Generally, the fjord's hydrography alternates seasonally between the dominance of cold Arctic and warm Atlantic water masses, depending on the strength of the West Spitsbergen Current (e.g. Svendsen et al. 2002, Hop et al. 2002a). Both transport characteristic planktonic fauna into the ecosystem (e.g. Hop et al. 2002a, Basedow et al. 2004, Hop et al. 2006, Buchholz et al. 2010). In recent years, however, observations indicated an increased input of Atlantic waters to the Arctic, and a year-round advection of Atlantic water into Kongsfjorden, which have been attributed to climate change (Falk-Petersen et al. 2007, Karcher et al. 2003, Walczowski et al. 2012). As a result, in addition to the previously prevailing arcto-boreal species *Thysanoessa inermis* and *T. raschii*, two boreal (*T. longicaudata* and *Meganyctiphanes norvegica*) and one subtropical-boreal krill species (*Nematoscelis megalops*) were recently found regularly in Kongsfjord (Buchholz et al. 2010).

Krill (Euphausiacea) are macrozooplankton and occupy a central trophic position in pelagic food webs throughout the world oceans by directly linking primary production to higher trophic levels with high efficiency (Falk-Petersen et al. 1990, Ellingsen et al. 2006, Dalpadado and Mowbray 2013). In polar regions, local lipid rich krill species are indispensable for many sea birds, (economically important) fish and marine mammals such as seals (e.g. Gjøsæter et al. 2002, Dalpadado and Bogstad 2004, Dolgov et al. 2010, Lindstrøm et al. 2013). The lipid

composition may vary remarkably between zooplankton species from different latitudes depending on the individuals' adaptation to survive in their specific environment of origin (Lee et al. 2006, Kattner and Hagen 2009). At high latitudes, the zooplankton community is adapted to store significant amounts of lipids to preserve metabolic energy from phytoplankton blooms to overcome long periods of food scarcity during polar night (Gradinger 1995, Falk-Petersen et al. 1981, Kattner and Hagen 2009). The arcto-boreal krill species *T. inermis* is particularly characterized by high lipid content and the capability to biosynthesize energy-rich wax esters as the main storage lipid, and therefore believed to be the most pronounced Arctic-adapted krill species (Falk-Petersen et al. 2000). In contrast, *T. raschii* and the more boreal species *T. longicaudata* and *M. norvegica* are known to primarily store their energy as triacylglycerols (Falk-Petersen et al. 2000).

Krill are predominantly omnivorous (Mauchline 1980) with specific preferences to a more herbivorous (e.g. *T. inermis* and *T. raschii*) or carnivorous diet (e.g. *T. longicaudata* and *M. norvegica*; e.g. Sargent and Falk-Petersen 1981). Feeding habits, seasonal lipid compositions and general life cycle characteristics of these four North Atlantic krill species have been reviewed by Falk-Petersen et al. (2000). However, the authors referred to investigations conducted more than 30 years ago (samplings between 1977 and 1983) which did not yet include *N. megalops*. According to Zhukova et al. (2009) this species was believed to be absent from these areas before the end of 1970. More recent research with regard to lipid content and trophic relationships of euphausiids from high latitude regions is available from samples taken in the waters surrounding Iceland (Petursdottir et al. 2008 and 2012), the Bering Sea (Harvey et al. 2012) and the Gullmarsfjord (Pond et al. 2012). But also, these studies do not consider the expatriate subtropical-temperate *N. megalops*. To date, lipid data on *N. megalops* are mainly restricted to the South Atlantic Ocean i.e. the Agulhas Current (Mayzaud et al. 2007, Richoux 2011), the Mediterranean Sea northwest of Mallorca (Cartes 2011) and the Sargasso Sea (Boyd et al. 1978). In these areas, the species shows a carnivorous feeding mode and a very low total lipid content mainly stored as polar lipids.

In summary, earlier investigations on the lipid composition of the five different krill species were only conducted in the areas of their typical biogeographic distribution. Nonetheless, despite the ecological role of euphausiids in the pelagic food web, there are to date, no studies comparing the individual feeding behaviour and biochemical composition of all the five species presently found in the high Arctic. Nevertheless possible adaptational differences in the species' biochemical composition may result in considerable ecological changes within the pelagic food web by altering the efficiency of energy transfer to higher trophic levels; e.g. through different lipid quality and quantity (Falk-Petersen et al. 2007, Kattner and Hagen 2009, Harvey et al. 2012). Furthermore, the increased species diversity may lead to an enhanced interspecific competition on shared food sources (Gradinger 1995) – particularly with other meso- and macrozooplankton such as calanoid copepods and fish larvae. Equally, the boreal and subtropical-boreal krill species recently expatriated to the high Arctic Kongsfjord will also have to cope with a different nutritional environment compared to their former foraging habitat.

Accordingly, our study was performed to elucidate the species-specific trophic interactions, feeding modes and lipid compositions within the changed krill community recently present in Kongsfjord. We further aimed at investigating the allocation of energy reserves through total lipid and lipid class analyses, in order to predict each species' potential to persist in this challenging environment. Furthermore, we investigated the trophic relationships and foraging habits by the analysis of fatty acid and stable isotope composition. For a fjord comparison, i.e. with reference to the lipid biochemical composition of the arcto-boreal *T. inermis*, samples were also taken from the Hornsund fjord (76°N), which is, compared to the Kongsfjord, a true

cold Arctic fjord with little influence of warmer Atlantic waters (Walczowski 2013). Additionally, we presented lipid data on *N. megalops* sampled at two different locations in the North and South Atlantic for a latitudinal comparison. The data collected in this study may be integrated into ecological modelling studies on complex food-web dynamics - especially within the Kongsfjord ecosystem.

4.2 Material and methods

4.2.1 Sample collection

Adult krill were sampled during four expeditions in 2006, 2011 and 2012. *Thysanoessa inermis* from Hornsund (July 2012) were sampled during the research cruise AREX2012 on-board the Polish RV *Oceania*. Krill from Kongsfjord (*Thysanoessa* spp., *Meganyctiphanes norvegica* and *Nematoscelis megalops*) were collected in 2006 and 2012 on-board the Kings Bay AS workboat MS *Teisten*. Further specimens of *N. megalops* were caught in 2011, on-board the German RV *Maria S. Merian* during leg 17/3 in the Benguela Current off the coast of Namibia, and in 2012, on-board the German RV *Meteor* during leg M87.1b in the Iceland Basin. For more details see Table 4.1 and Fig. 4.1.

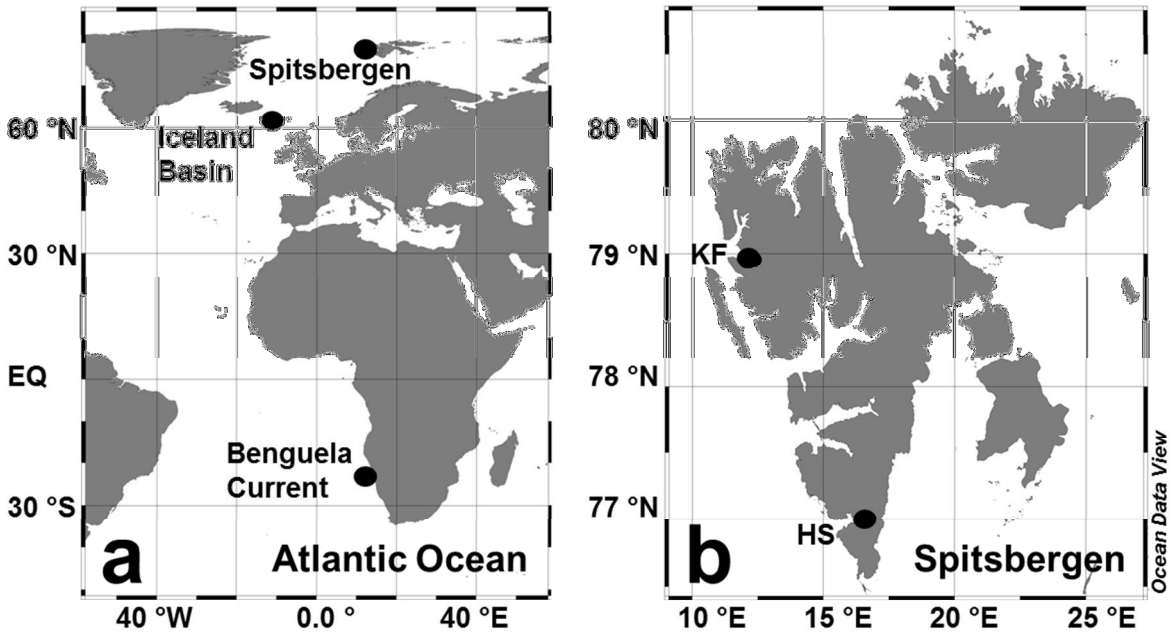

Fig. 4.1 *Station maps with sampling locations. (a) Overview of the Atlantic Ocean with stations sampled for latitudinal comparison. (b) Map of Spitsbergen with dots highlighting the position of the two fjords sampled. HS= Hornsund, KF = Kongsfjord. Detailed station positions are shown in Table 4.1.*

Trawls were operated at a speed of two knots using a 1 m^2 Tucker trawl (1000 µm mesh size and soft cod-end bucket). *N. megalops* sampled in the Benguela Current and in the Iceland Basin were caught operating a 1 m^2 MOCNESS (Wiebe et al. 1976) equipped with nine nets (2000 µm mesh size and soft cod-end net bucket).

Specimens captured were transferred to aerated aquaria filled with filtered seawater and given a minimum of 12 h acclimation period at in-situ temperature in dim light before sex and size (from the front of the eyes to the tip of the telson to the nearest mm) determination under a stereomicroscope. The specimens were then rinsed with distilled water, shock frozen in liquid nitrogen and stored at -80°C for later analysis. Except for *T. inermis*, only male specimens were sampled for lipid analyses to minimize reproductive influences on lipid composition (e.g. Clarke 1980).

Table 4.1 *List of species investigated, station names, sampling positions and dates of catch. See also Fig. 4.1.*

Species	Location	Station	Latitude	Longitude	Date of catch
T. inermis	Kongsfjord	KF IF (I)	78.96°N	12.37°E	28.07.2006
	Hornsund	HS	76.99°N	16.33°E	30.07.2012
	Kongsfjord	KF IF (I)	78.96°N	12.37°E	22.08.2012
T. raschii	Kongsfjord	KF IF (II)	78.97°N	12.33°E	24.08.2012
T. longicaudata	Kongsfjord	KF IF (I)	78.96°N	12.37°E	22.08.2012
M. norvegica	Kongsfjord	KF NyÅle	78.95°N	12.05°E	17.08.2012
N. megalops	Kongsfjord	KF IF (II)	78.97°N	12.33°E	24.08.2012
	Iceland Basin	M 1.3	61.50°N	11.00°W	29.04.2012
	Benguela Current	WLT-2a	23.53°S	12.98°E	31.01.2011

4.2.3 Lipid analyses

Whole animals were lyophilized for 24 h and their dry weights determined. Lipids were extracted after Folch et al. (1957) and the total lipid content was identified gravimetrically (Hagen 2000). The total content was expressed as percent of dry weight (% DW). The extractions were performed in the laboratories of the department of Marine Zoology, University of Bremen (BreMarE, Germany).

The separation and identification of lipid classes were done at AWI (Bremerhaven) on the total lipid extracts according to Graeve and Janssen (2009) on a silica column (Chromolith®Performance-Si 100 x 4.6 mm i.d.) using the LaChromElite HPLC system (VWR, Darmstadt, Germany) with an SEDEX 40 evaporative light scattering detector. Lipid classes were identified according to a commercially available standard and were expressed as percent of total lipid.

Fatty acid and fatty alcohol compositions were identified simultaneously following the method of Kattner and Fricke (1986). Aliquots of the total lipid extracts were hydrolysed under nitrogen atmosphere in methanol containing 3 % concentrated sulphuric acid and transesterificated for 4 h at 80°C. Subsequent analyses were done by gas liquid chromatography (HP 6890N GC) on a wall-coated open tubular column (30 x 0.25 mm inside diameter; film thickness: 0.25 µm; liquid phase: DB-FFAP) using temperature programming. Standard mixtures were used to identify the fatty acid methyl esters and the fatty alcohols. When necessary, further identification was done by GC-mass spectrometry using a

comparable capillary column. Detailed fatty acid and fatty alcohol composition were expressed as percent of total fatty acid and accordingly percent of total fatty alcohols.

Commonly used fatty acid trophic markers, indicating the different food sources consumed, were applied to investigate the interspecies trophic relationships (e.g. Dalsgaard et al. 2003, Lee et al. 2006, Cartes 2011, Legezynska et al. 2014). 16:1(n-7), 18:1(n-7) and 20:5(n-3) fatty acid as biomarkers for diatoms, 16:0, 18:4(n-3) and 22:6(n-3) fatty acid as predominant markers for (dino-) flagellates, and 18:1(n-9), 20:1(n-9) and 22:1(n-9) fatty acid as indicators for carnivory and a copepod diet respectively.

Furthermore, fatty acid ratios were calculated giving additional information on trophic relationships. The ratio of total polyunsaturated fatty acids (PUFA) to saturated fatty acids (SFA) was used as an indication for carnivory (e.g. Cripps and Atkinson 2000). Ratios of 16:1(n-7)/16:0, 16:1n-7/18:4(n-3) and 20:5(n-3)/22:6(n-3) (e.g. Graeve et al. 1994, Nelson et al. 2001, Reuss and Poulsen 2002) were calculated, in order to differentiate between a diatom and a flagellate based nutrition. Although giving the same information, the frequently used quotient of the fatty acid moieties 18:1(n-9) and 18:1n-7 (e.g. Graeve et al. 1997, Nelson et al. 2001, Auel et al. 2002) was considered not to be appropriate in interspecies comparison as these are known to be *de novo* biosynthesized by *Thysanoessa* spp. (Falk-Petersen et al. 2000).

4.2.4 Stable isotope analyses

Stable isotope ratios were analysed on muscle tissue (as prioritized by e.g. Søreide et al. 2006b) taken from krill species sampled at the stations in Hornsund and Kongsfjord, Spitsbergen (Table 4.1, Fig. 4.1). The samples were lyophilized for 24 h, weighed into tin capsules and analysed by Agroisolab GmbH (TÜV Rheinland Group; Jülich, Germany). The ratios were expressed using the equation δX (‰) = $[(R_{Sample} / R_{Standard}) - 1] \times 1000$, where δX was the ^{13}C or ^{15}N value of the sample, R_{Sample} the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the sample and $R_{Standard}$ the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the reference standards: Vienna Pee Dee Belemnite (VPDB) was used for carbon and atmospheric nitrogen (air) for nitrogen, respectively. Standards of the International Atomic Energy Agency (IAEA) were applied for the calibration during isotopic mass spectroscopy and inserted every 12th sample. Polyethylene (IAEA-CH7; $\delta^{13}C = -32.2$‰) and sucrose (IAEA-CH6; $\delta^{13}C = -10.4$‰) were used for the calibration of $\delta^{13}C$. Ammonium sulphate (IAEA-N1; $\delta^{15}N = 0.4$‰ and IAEA-N_2; $\delta^{15}N = 20.3$‰) were used for the calibration of $\delta^{15}N$. Trophic levels (Tl) were calculated by modifying the relationship used by Fisk et al. (2001) assuming the trophic enrichment factor of the European Arctic (3.4 ‰) determined by Søreide et al. (2006a): $Tl_{Krill} = Tl_{Calanus} + (\delta^{15}N_{Krill} - \delta^{15}N_{Calanus}) / 3.4$. In our study, *Calanus* copepods were set to trophic level 2 as they generally represent the first consumers on phytoplankton (e.g. Fisk et al. 2001, Hop et al. 2002b, Nilsen et al. 2008, Wold et al. 2011) and used as the baseline value for calculation ($\delta^{15}N_{Calanus}$). Based on spring/summer data of *Calanus* from Kongsfjord, this value was set to 6.7 ‰ according to Wold et al. (2011).

The feeding strategy for each krill species was estimated according the feeding categories listed in Søreide et al. (2006a): Tl ≤ 2.3 = predominant herbivorous, Tl 2.4 – Tl 2.8 = predominant omnivorous and Tl 2.9 – Tl 3.3 = predominant carnivorous feeding.

4.2.5 Statistical methods

All data were given as means ± standard deviation unless specified otherwise. The level of significance was set at $p < 0.05$. Differences in stable isotopic signatures of the krill species were tested by one-way ANOVA. The same test was used to test for differences on the

species-specific mean fatty acid ratios. All univariate statistics were performed and displayed using the statistical software package GraphPad Prism5 (GraphPad Software, Inc., USA). Multivariate statistics were performed on fatty acid moiety compositional data using the PAST software package version 3.01 (Hammer et al. 2001). The data-sets were left untransformed according to Howell et al. (2003). A one-way PERMANOVA (permutational multivariate analysis of variance based on chi-squared distances) was used to test for statistical significances among species. SIMPER (similarity percentage analysis based on chi-squared distances) was used in order to identify the contribution of certain fatty acids to average dissimilarity between species. The similarity pattern within the fatty acid trophic markers found in the species from Kongsfjord was visualized by correspondence analysis (Greenacre and Primicerio 2013). Correspondence analysis has been shown to follow closely the principle of compositional coherence, which is important in analysing component data (Greenacre 2011).

4.3 Results

All specimens of *Thysanoessa* spp. sampled at Spitsbergen were adult but sexually non-active. Concerning *Thysanoessa inermis*, stable isotope analysis showed no significant differences between sexes, years of sampling (i.e. 2006 and 2012) and locations of catch (i.e. Kongsfjord and Hornsund; tested by one-way ANOVA). Therefore, these samples were pooled for interspecies comparison. The same was found for lipid compositional data. These data were only pooled with regard to sex and years of sampling and shown for each fjord (e.g. Table 4.2). In the following, unless explicitly mentioned, the statements on "*T. inermis*" refer to specimens sampled from both fjords.

Table 4.2 *Proximate data (n = number of individuals) of Thysanoessa inermis (Ti KF), T. raschii (Tr), T. longicaudata (TL), Meganyctiphanes norvegica (Mn) and Nematoscelis megalops (Nm) sampled in Kongsfjord and of T. inermis sampled in Hornsund (Ti HS). Values are given as means ± standard deviations.*

	Ti HS (n = 7)	*Ti* KF (n = 13)	*TL* (n = 3)	*Tr* (n = 3)	*Mn* (n = 3)	*Nm* (n = 4)
Length (mm)	24.3 ± 1.0	23.5 ± 1.7	14.7 ± 2.8	23.0 ± 2.6	30.0 ± 1.0	22.5 ± 0.7
Dry weight (mg)	30.4 ± 7.1	30.7 ± 4.6	6.4 ± 4.4	18.0 ± 4.2	46.1 ± 6.7	14.2 ± 0.9
Total lipid (mg)	14.0 ± 3.7	13.3 ± 2.5	4.0 ± 1.1	5.2 ± 1.8	13.3 ± 1.6	1.5 ± 0.3
Total lipid (% dry	46.2 ± 6.5	43.4 ± 4.2	43.5 ± 6.5	28.5 ± 4.0	28.9 ± 2.2	10.4 ± 1.4

4.3.1 Biometry and total lipid content

With a mean length of 14.7 mm, *T. longicaudata* was the smallest of the five krill species sampled, followed by *Nematoscelis megalops*, *T. raschii* and *T. inermis* with mean body lengths ranging from 22.5 to 24.3 mm (Table 4.2). *Meganyctiphanes norvegica* was the largest and heaviest species with a mean length of 30.0 mm and a mean body dry weight of ~ 46 mg. *T. longicaudata* had the lowest mean body dry mass of ~ 6 mg. Despite almost the same body size, *T. inermis* had a much higher mean body dry mass (~ 30 mg) than *T. raschii* (~ 18 mg) and *N. megalops* (~ 14 mg). Highest total lipid mass (> 10 mg per individual) was found in *T. inermis* and *M. norvegica,* which was about double the mass of *T. raschii* and *T. longicaudata* and almost ten times higher than that of *N. megalops* (~ 1.5 mg per

individual). However, taking the species' individual body masses into account, highest mean total lipid contents were found in *T. inermis* (Table 4.2; range 35 – 54 % DW) and *T. longicaudata* (Table 4.2; 37 – 52 % DW) whereas *T. raschii* and *M. norvegica* showed only intermediate mean total lipid contents of about 28 % DW (Table 4.2; range 24 – 31 % DW). With 10.4 % DW, *N. megalops* had the lowest mean total lipid content of the specimens sampled in Kongsfjord (Table 4.2; range 9 – 12 % DW).

Lipid class composition

In *T. inermis* the major lipid class were wax esters accounting for 43.2 ± 3.4 % of total lipid in specimens from Hornsund and 49.0 ± 3.4 % in specimens from Kongsfjord (Fig. 4.2; Online Resource 1). In contrast, the lipid of the other species investigated contained only low levels of wax esters (*T. longicaudata*, *T. raschii* and *N. megalops*; on average 2.6 – 8.9 % total lipid) to almost no wax esters (*M. norvegica*). Triacylglycerols were dominating the lipids of *M. norvegica* (67.5 ± 2.7 %), *T. raschii* (47.3 + 9.6 %) and *T. longicaudata* (51.8 ± 1.4 %). Triacylglycerols were also present in *T. inermis* but in relatively low quantities (21.3 ± 2.8 % for *T. inermis* from Hornsund and 17.2 ± 4.8 % for *T. inermis* from Kongsfjord). The lipids of *N. megalops* contained only low amounts of triacylglycerols (5.8 ± 2.4 %). Wax ester contents were also low (8.9 ± 2.3 %). In contrast, the lipids of *N. megalops* were clearly dominated by the polar lipids phosphatidylethanolamine (26.3 ± 2.8 %) and phosphatidylcholine (47.4 ± 2.2 %), and the species also had relatively high mean contents of sterols (13.1 ± 0.7 %).

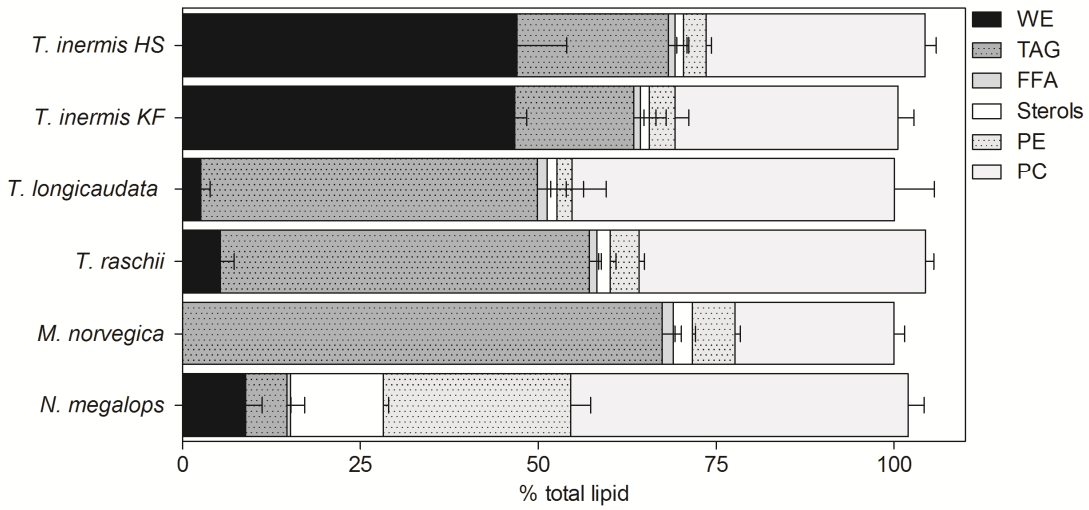

Fig. 4.2 *Lipid class composition of the species sampled in Spitsbergen (see also Online Resource 1). WE = Wax ester; TAG = Triacylglycerol; FFA = Free fatty acids; PE = Phosphatidylethanolamine; PC = Phosphatidylcholine. Compositions are shown as the mean percentages of total lipid ± standard deviations (n = 3 – 13; Table 4.2).*

4.3.2 Fatty acid and alcohol composition

As a consequence of its wax ester content, *T. inermis* had the highest mean proportion of fatty alcohols (14.4 ± 0.6 % of total lipid in specimens from Hornsund and 15.7 ± 1.0 in specimens from Kongsfjord; Table 4.3). In *T. raschii, T. longicaudata* and *N. megalops* fatty alcohols were only present in small amounts (< 4 % of total lipid). For *M. norvegica* no fatty alcohols were detectable. The short chain fatty alcohols 14:0, 16:0 and 16:1(n-7) were only

present in the *Thysanoessa* species and not found in *N. megalops*, which only contained long chain alcohols 20:1(n-9) and 22:1(n-11) (with minor amounts of other isomers) typical for *Calanus* wax esters. They were also present in traces in *T. inermis* and *T. raschii* but absent in *T. longicaudata*.

Fatty acid and alcohol analyses of total lipids showed that 17 fatty acids accounted for > 1 % in the five krill species (shown in Table 4.3). The 22:6(n-3) fatty acid was dominant in *N. megalops* (24.3 ± 0.5 %; Table 4.3), whereas the fatty acids of *T. inermis* were dominated by 18:1(n-9) (24.4 ± 3.9 % in specimens from Hornsund and 21.6 ± 2.4 in specimens from Kongsfjord). The 22:1(n-11) fatty acid was most abundant in *M. norvegica* (9.6 ± 3.2 %) and was reduced in the other species (< 1 – 3 %; Table 4.3). Palmitic acid (16:0) was relatively more abundant in the *Thysanoessa* species, especially in *T. longicaudata* (33.1 ± 0.9 %; Table 4.3), compared to *N. megalops* (18.6 ± 0.9 %) and *M. norvegica* (15.3 ± 0.4 %).

PERMANOVA analysis showed that the differences in fatty acid composition between the species were significant ($p < 0.0001$, $F = 26.9$). SIMPER analysis distinguished nine fatty acids [all of them commonly used fatty acid trophic markers (see Material and Methods section)] contributing to > 80 % of the average dissimilarity between species: the saturated fatty acid 16:0; the monounsaturated fatty acids 16:1(n-7), 18:1(n-9), 18:1(n-7), 20:1(n-9) and 22:1(n-11); and the polyunsaturated fatty acids 18:4(n-3), 20:5(n-3) and 22:6(n-3). The 20:1(n-9) isomer was the main fatty acid responsible for differentiation contributing 20.5 % to the average dissimilarity followed by 22:6(n-3) and 22:1(n-11) fatty acid contributing 18.8 % and 15.6 %, respectively. The contributions of the remaining fatty acids were < 10 %.

The variation between the nine fatty acid trophic markers related to the different krill species was visualized by correspondence analysis (Fig. 4.3), which clearly separated the species into three groups: the *Thysanoessa* species, *N. megalops* and *M. norvegica*. Compared to the other species, the three *Thysanoessa* species were grouped together being characterized by higher levels of 18:4(n-3), 18:1(n-9) and 18:1(n-7) fatty acids. Within this group *T. longicaudata* contained comparatively high amounts of 16:0 (see above) and lower amounts of 20:5 (n-3) (10 ± 0.6 %; Fig. 4.3; Table 4.3). *T. raschii* was more associated to 16:1(n-7) and showed the highest contents of the long chain moieties 20:1(n-9) and 22:1(n-11) fatty acid (3.0 ± 0.7 % and 2.0 ± 0.4 %, respectively). *T. inermis* from both fjords grouped together, having the highest amounts of 18:1(n-9) and 18:1(n-7) moieties (Table 4.3). Nevertheless, compared to the specimens sampled in Kongsfjord, the specimens from Hornsund were less associated to the long chain fatty acids 20:1(n-9) and 22:1(n-11) and more enriched in 18:4(n-3) (5.6 ± 2.9 % compared to 2.5 ± 1.0 %). Correspondence analysis also showed the afore mentioned high contents of 22:6(n-3) in the total fatty acids of *N. megalops* (~ 25 %) and concurrently highlighted the negative correlation to the 16:1(n-7), 18:1(n-9) and 18:4(n-3) fatty acids leading to a grouping opposite to the *Thysanoessa* species (Fig. 4.3). *M. norvegica* formed the third group, having the lowest 16:0 and 18:1(n-9) contents and the highest proportion in 20:1(n-9) and 22:1(n-11). It diverged from the *Thysanoessa* species to the negative side of axis 1 and due to comparable low 22:6(n-3) abundances (~ 10 %) to the positive side of axis 2 of *N. megalops* (Fig. 4.3; Table 4.3).

Table 4.3 *Fatty acid and alcohol composition (% of total fatty acids and alcohols) of Thysanoessa inermis (Ti KF), T. raschii (Tr), T. longicaudata (TL), Meganyctiphanes norvegica (Mn) and Nematoscelis megalops (Nm) and of T. inermis sampled in Hornsund (Ti HS). Values are given as mean percentages ± standard deviations. Compounds < 1 % are not shown or marked as traces (tr) as soon as they were present in comparative species.(n = number of individuals; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids).*

	Ti HS	*Ti* KF	*TL*	*Tr*	*Mn*	*Nm*
	(*n* = 7)	(*n* = 13)	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 4)
Fatty acid						
14:0	2.2 ± 0.5	2.3 ± 0.4	3.4 ± 0.6	6.0 ± 0.7	4.8 ± 0.2	1.7 ± 0.4
16:0	22.1 ± 0.8	21.4 ± 1.2	33.1 ± 0.9	26.8 ± 1.1	15.3 ± 0.4	18.6 ± 0.9
18:0	1.8 ± 0.3	1.4 ± 0.4	2.0 ± 0.2	2.1 ± 0.2	1.3 ± 0.2	tr
Σ SFAs	26.0 ± 0.8	25.0 ± 1.4	38.5 ± 1.0	34.9 ± 1.1	21.4 ± 0.4	21.0 ± 0.9
16:1(n-7)	7.9 ± 3.4	8.8 ± 3.2	7.5 ± 2.1	12.6 ± 1.0	6.2 ± 0.5	2.7 ± 0.3
18:1(n-9)	24.4 ± 3.9	21.6 ± 2.4	17.7 ± 1.0	11.9 ± 1.2	9.1 ± 0.7	12.5 ± 0.3
18:1(n-7)	10.7 ± 1.8	11.0 ± 1.8	7.6 ± 0.1	9.5 ± 1.1	4.9 ± 0.6	4.2 ± 0.4
20:1(n-9)	1.2 ± 0.6	2.5 ± 1.7	2.7 ± 0.5	3.0 ± 0.7	17.3 ± 0.4	6.5 ± 0.8
20:1(n-7)	tr	tr	tr	tr	1.1 ± 0.2	tr
22:1(n-11)	tr	1.2 ± 1.1	tr	2.0 ± 0.4	9.6 ± 3.2	3.0 ± 0.5
22:1(n-9)	-	tr	tr	tr	1.6 ± 0.2	tr
Σ MUFAs	45.3 ± 1.7	46.2 ± 3.4	38.3 ± 2.1	41.0 ± 2.5	50.9 ± 2.4	30.4 ± 1.2
16:2(n-4)	tr	tr	tr	1.2 ± 0.1	tr	-
18:2(n-6)	1.0 ± 0.3	1.1 ±0.3	1.4 ± 0.3	tr	1.5 ± 0.3	3.5 ± 0.2
18:3(n-3)	tr	tr	tr	tr	tr	1.6 ± 1.0
18:4(n-3)	5.6 ± 2.9	2.5 ± 1.0	1.9 ± 0.9	1.1 ± 0.5	2.5 ± 0.4	tr
20:4(n-6)	tr	tr	tr	tr	tr	1.3 ± 0.1
20:5(n-3)	14.5 ± 2.5	16.1 ± 1.2	10.0 ± 0.6	12.8 ± 1.3	10.1 ± 0.8	15.8 ± 0.5
22:6(n-3)	5.1 ± 0.4	5.4 ± 1.1	7.8 ± 0.5	6.5 ± 1.2	10.5 ± 1.6	24.3 ± 0.5
Σ PUFAs	28.2 ± 1.1	27.0 ± 2.2	23.2 ± 1.5	24.1 ± 2.7	27.7 ± 2.0	48.6 ± 0.7
Fatty alcohol						
14:0	30.3 ± 3.5	27.4 ± 3.5	tr	tr	-	-
16:0	57.9 ± 3.3	53.8 ± 8.4	tr	tr	-	-
16:1(n-7)*	11.7 ± 0.5	12.7 ± 1.5	tr	tr	-	-
20:1(n-9/n-7)	tr	tr	-	tr	-	tr
22:1(n-11/n-9)	tr	tr	-	tr	-	tr
Ratios						
16:1(n-7) / 16:0	0.4 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	0.5 ± 0.0	0.4 ± 0.0	0.1 ± 0.0
16:1(n-7) / 18:4(n-	2.2 ± 1.8	4.0 ± 1.8	5.4 ± 4.7	13.1 ± 6.5	2.6 ± 0.3	5.1 ± 2.4
20:5(n-3) / 22:6(n-	2.9 ± 0.7	3.1 ± 0.8	1.3 ± 0.1	2.0 ± 0.3	1.0 ± 0.1	0.7 ± 0.0
PUFA / SFA	1.1 ± 0.0	1.1 ± 0.1	0.6 ± 0.0	0.7 ± 0.1	1.3 ± 0.1	2.3 ± 0.1

*might coincide with small amounts of 16:4(n-1) fatty acid

The fatty acid ratios calculated from certain fatty acids are commonly used to determine differences in food sources (for details see Materials and Methods) and were significantly different between species (see below; one-way ANOVA). Both the 16:1(n-7)/16:0 ($p = 0.0072$; F = 4.1) and the 20:5(n-3)/22:6(n-3) ($p < 0.0001$; F = 15.5) ratios showed lowest levels in *N. megalops* (~ 0.1 and ~ 0.7, respectively; Table 4.3). Within the *Thysanoessa* species, these values were lowest in *T. longicaudata* (~0.2 and ~ 1.3, respectively) and highest in *T. inermis* (~ 0.4 and ~ 3.0) followed by *T. raschii* (~ 0.5 and ~ 2.0). The 16:1(n-7)/18:4(n-3) ratio was characterized by relatively high standard deviations (Table 4.3). Nevertheless, the differences were significant between species ($p = 0.0002$; F = 7.3). Highest values were found for *T. raschii* (~ 13.1), *T. longicaudata* (~ 5.4) and *N. megalops* (~ 5.1). The ratio PUFA/SFA, showed highest values in *N. megalops* (~ 2.3), followed by *M. norvegica* (~ 1.0) and *T. inermis* (~ 1.3) having similar but lower values and *T. raschii* (~ 0.7) and *T. longicaudata* (~ 0.6) having the lowest values ($p < 0.0001$; F = 216.5).

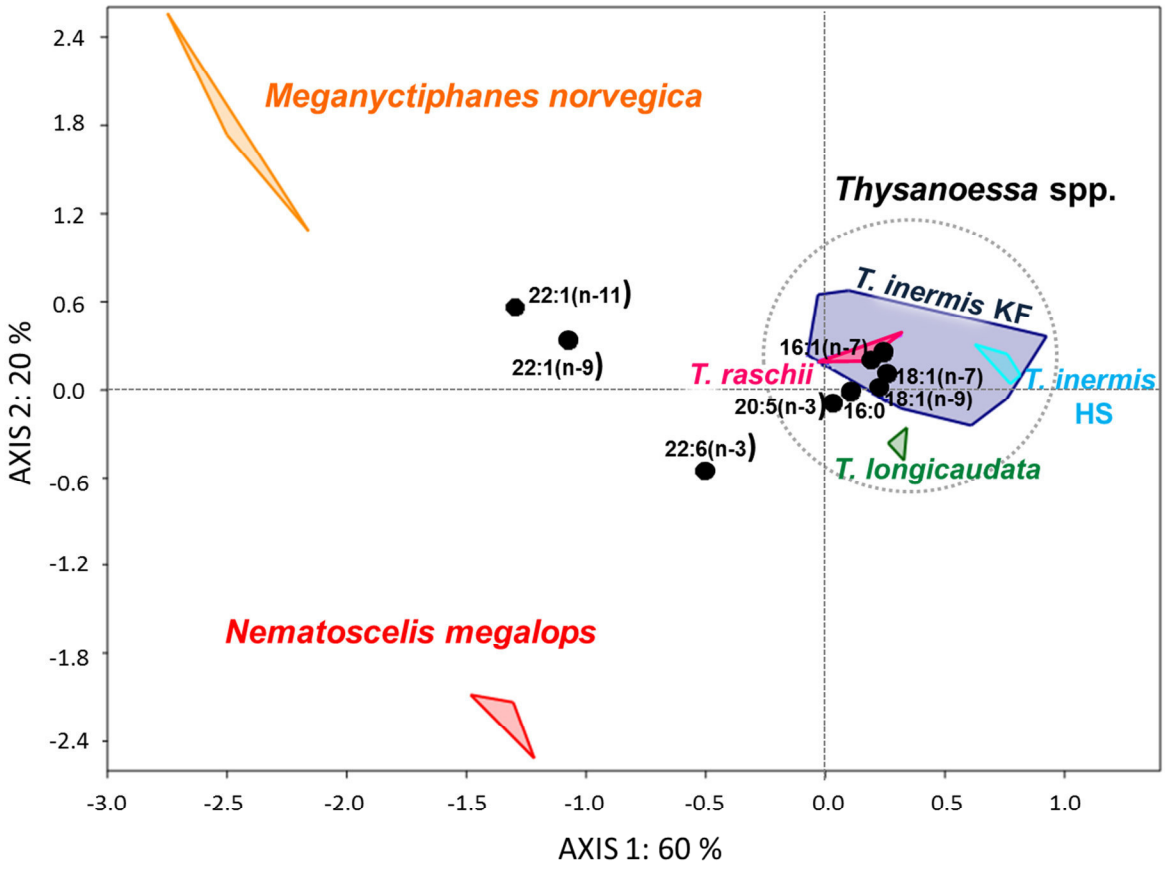

Fig. 4.3 *Correspondence analysis relating the variation in the fatty acid trophic marker composition (black dots) to the different krill species (coloured convex hulls, see Greenacre 2006; n = 3 – 13, see Table 4.2) sampled in Kongsfjord (KF) and in Hornsund (i.e. Thysanoessa inermis HS). The ordination biplot is based on the first two axes explaining 80 % of the total variance in the fatty acid trophic marker data.*

Stable isotope analyses

The isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) showed significant variations between species ($\delta^{13}C$: $p < 0.0001$, F = 8.1; $\delta^{15}N$: $p < 0.0001$, F = 9.1; Fig. 4.4a). Accordingly, calculated trophic levels were significantly different ($p < 0.0001$, F = 10.2; Fig. 4.4b). Overall, all krill species were situated between the trophic levels two and three. *N. megalops* occupied the highest mean tropic level of 2.9 (mean $\delta^{15}N$ = 9.8 ‰), which is almost one trophic level above that of *Calanus* species indicating predominantly carnivorous feeding. The lowest mean trophic level of 2.4, indicating predominant omnivorous feeding, was found in *T. longicaudata* with an average $\delta^{15}N$ of 8.0 ‰. *T. raschii*, *M. norvegica* (both having a mean trophic level of 2.6) and *T. inermis* (mean trophic level of 2.7) were situated between these species and therefore defined as omnivores, too (Fig. 4.4b; for the definition of the thresholds see Material and Methods 'Stable isotope analyses'). Regarding the carbon isotope ratios, *M. norvegica* showed the highest mean values (-20.78 ‰) followed by *N. megalops* (-21.06 ‰), *T. raschii* (-21.31 ‰), *T. inermis* (-21.47 ‰) and *T. longicaudata* (-22.22 ‰).

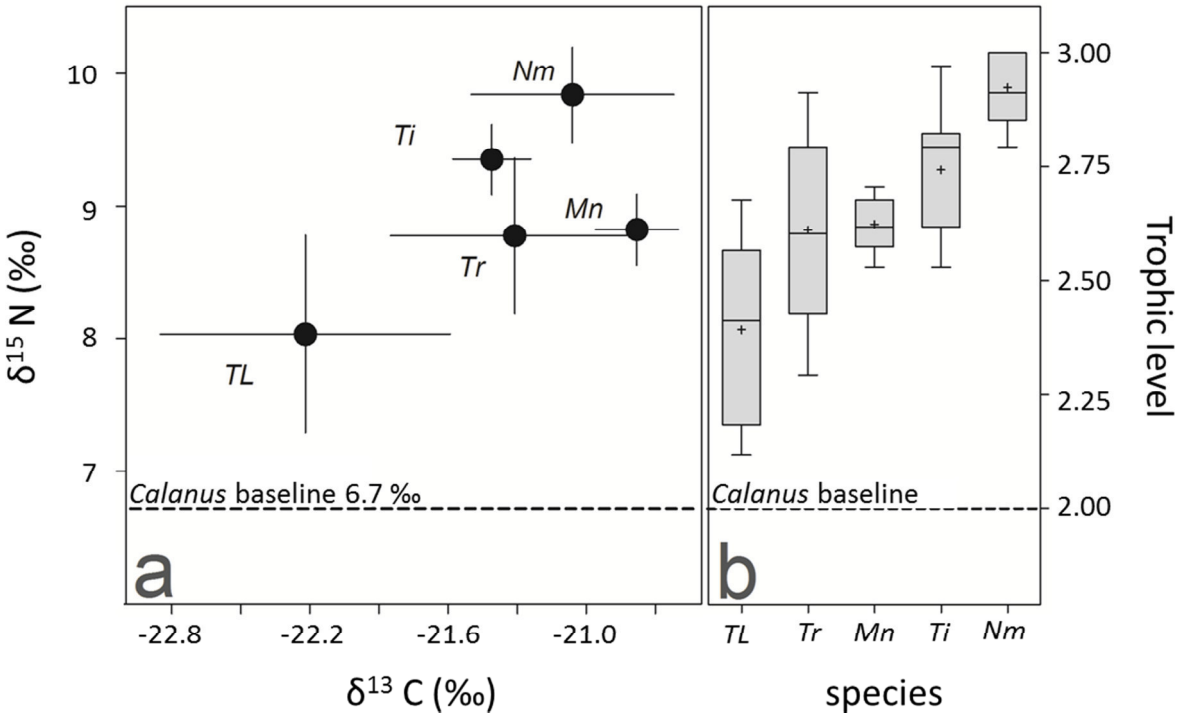

Fig. 4.4 *Stable isotopes and trophic levels of Thysanoessa inermis (Ti, n = 21), T. raschii (Tr, n = 8), T. longicaudata (TL, n = 6), Meganyctiphanes norvegica (Mn, n = 5) and Nematoscelis megalops (Nm, n = 5) sampled on Spitsbergen. (a) Stable carbon ($\delta^{13}C$) and stable nitrogen ($\delta^{15}N$) values given as means ± 95 % confidence intervals. (b) Calculated trophic levels shown in a boxplot giving the median with quartiles. Whiskers are pointing to minimum and maximum. Crosses indicate the mean trophic level. [n = number of individuals; Calanus baseline for Kongsfjord was taken from Wold et al. (2011)].*

4.3.3 Latitudinal comparison in Nematoscelis megalops

Table 4.4 *Proximate data and lipid class composition (n = number of individuals) of Nematoscelis megalops sampled in the high Arctic Kongsfjord, Iceland Basin and northern Benguela Current. Values are given as means ± standard deviations.*

	Kongsfjord (n = 4)	Iceland Basin (n = 3)	Benguela Current (n = 3)
Length (mm)	22.5 ± 0.7	20.7 ± 0.6	17.0 ± 0.0
Dry weight (mg)	14.2 ± 0.9	12.0 ± 0.5	7.4 ± 0.5
Total lipid (mg)	1.5 ± 0.3	1.1 ± 0.0	0.6 ± 0.1
Total lipid (% dry weight)	10.4 ± 1.4	9.6 ± 0.4	7.6 ± 0.9
Lipid class (% total lipid)			
Wax ester	8.9 ± 2.3	11.7 ± 0.8	1.8 ± 1.7
Triacylglycerol	5.8 ± 2.4	6.0 ± 1.5	4.9 ± 3.8
Sterols	13.1 ± 0.7	11.9 ± 1.1	21.6 ± 2.4
Free fatty acids	tr	tr	1.5 ± 0.6
Phosphatidylethanolamine	26.3 ± 2.8	22.0 ± 1.6	22.2 ± 3.4
Phosphatidylcholine	47.4 ± 2.2	48.0 ± 1.0	47.9 ± 0.7

For supplementary investigation on the lipid profile of *N. megalops*, samples were analysed from the Iceland Basin (North Atlantic Ocean; south of Iceland) and the Benguela Current (South Atlantic Ocean; west coast of Namibia; Fig. 4.1a), Table 4.1). With regard to mean body length, dry weight and total lipid content, specimens from the Iceland Basin were similar to those sampled in Kongsfjord (Table 4.4). In comparison, *N. megalops* sampled in the Benguela Current were significantly smaller and therefore lower in dry weight ($p < 0.0001$, F = 57.6 and 85.7 respectively). The lipid content was also significantly lower (Table 4; $p = 0.027$, F = 6.3) and mainly consisted of sterols (21.6 ± 2.4 %) and the polar lipids phosphatidylethanolamine (22.2 ± 3.4 %) and phosphatidylcholine (47.9 ± 0.7 %). Polar lipids also dominated the total lipids of the specimens from Kongsfjord (~ 26 % and ~ 47 %, respectively) and Iceland Basin (~ 22 and ~ 48 %, respectively). Nevertheless, within the total lipids, these animals showed significantly lower amounts of sterols (~ 13 % for specimens from Kongsfjord and ~ 12 % for specimens from Iceland basin; $p = 0.0002$, F = 39.2) and higher amounts of wax esters (~ 9 % for specimens from Kongsfjord and ~ 12 % for specimens from Iceland basin; $p = 0.001$, F = 26.6) than the specimens from the Benguela Current (~ 2 % wax ester). Similarly, fatty alcohols were not found in *N. megalops* from the Benguela Current but were present in the specimens sampled in the other regions (solely composed of long chained 20:1 and 22:1 alcohols; Table 4.5).

Overall, 13 fatty acids were found for *N. megalops* (Table 4.5) contributing to > 1 % to the total composition. The typical membrane bound fatty acids16:0, 18:1(n-9), 20:5(n-3) and 22:6(n-3) were detected in highest amounts in all specimens. The long chain fatty acids 20:1(n-9) and 22:1(n-11) were present in higher abundances in specimens from the Kongsfjord and the Iceland Basin, (~ 7 % and ~ 3 %, respectively) compared to animals from the Benguela Current, in which 20:1(n-9) was found in traces and 22:1(n-11) was absent (Table 4.5). Fatty acid ratios were similar in the specimens sampled at the different locations. The same accounted for most of the fatty acid compositional data. However, multivariate data

analysis showed distinctions between locations (p = 0.0004, F = 20.4; PERMANOVA). SIMPER analysis identified five fatty acids cumulatively contributing ~ 80 % to average dissimilarity: 22:1(n-11), 20:1(n-9), 20:4(n-6), 18:1(n-9) and 22:6(n-3) (arranged from high to low percentaged contribution).

Table 4.5 *Fatty acid and alcohol composition (% of total fatty acids and alcohols) of Nematoscelis megalops sampled in the high Arctic Kongsfjord, Iceland Basin and northern Benguela Current. Values are given as mean percentages ± standard deviations. Compounds < 1 % are not shown or marked as traces (tr) as soon as they were present in comparative specimens.(n = number of individuals; SFAs = saturated fatty acids; MUFAs = monounsaturated fatty acids; PUFAs = polyunsaturated fatty acids).*

	Kongsfjord (n = 4)	Iceland Basin (n = 3)	Benguela Current (n = 3)
Fatty acid			
14:0	1.7 ± 0.4	2.2 ± 0.5	2.1 ± 1.2
16:0	18.6 ± 0.9	19.0 ± 1.1	22.1 ± 1.9
18:0	tr	1.4 ± 0.2	1.8 ± 0.0
Σ SFAs	21.0 ± 0.9	22.7 ± 0.9	26.0 ± 3.0
16:1(n-7)	2.7 ± 0.3	3.2 ± 0.3	2.4 ± 0.7
18:1(n-9)	12.5 ± 0.3	11.1 ± 0.8	15.6 ± 2.4
18:1(n-7)	4.2 ± 0.4	3.7 ± 0.1	3.8 ± 0.1
20:1(n-9)	6.5 ± 0.8	5.7 ± 0.9	tr
22:1(n-11)	3.0 ± 0.5	5.4 ± 1.2	-
Σ MUFAs	30.4 ± 1.2	30.4 ± 1.4	22.4 ± 2.2
18:2(n-6)	3.5 ± 0.2	1.6 ± 0.2	2.7 ± 0.1
18:3(n-3)	1.6 ± 1.0	tr	tr
20:4(n-6)	1.3 ± 0.1	1.5 ± 0.3	3.5 ± 0.5
20:5(n-3)	15.8 ± 0.5	19.0 ± 0.5	15.7 ± 0.2
22:6(n-3)	24.3 ± 0.5	22.7 ± 0.3	29.0 ± 2.2
Σ PUFAs	48.6 ± 0.7	46.9 ± 0.6	51.6 ± 2.2
Fatty alcohol			
20:1(n-9/n-7)	tr	tr	-
22:1(n-11/n-9)	tr	tr	-
Ratios			
16:1(n-7) / 16:0	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0
20:5(n-3) / 22:6(n-3)	0.7 ± 0.0	0.8 ± 0.0	0.5 ± 0.0
18:1(n-9) / 18:1(n-7)	3.0 ± 0.3	3.0 ± 0.2	4.1 ± 0.7
PUFA / SFA	2.3 ± 0.1	2.1 ± 0.1	2.0 ± 0.3

4.4 Discussion

In our study, we found significant interspecific differences between the five krill species from different geographical origin (Einarsson 1945); namely *Thysanoessa inermis* (arcto-boreal), *T. raschii* (arcto-boreal), *T. longicaudata* (boreal), *Meganyctiphanes norvegica* (boreal) and *Nematoscelis megalops* (subtropical-boreal) currently forming the euphausiid community within Kongsfjord. All lipid and fatty acid data were comparable to other studies; i.e. to those conducted in the specific areas of origin of the different krill species (see below). As a first conclusion, we assume all species sampled in normal, i.e. good nutritional condition not having suffered starvation, which otherwise would have been indicated by lower lipid contents and reduced whole-animal performance (Boyd et al. 1978, Huenerlage and Buchholz 2013, Huenerlage et al. in rev.).

The five krill species did not only differ in their total lipid content, but also in their lipid composition and specific trophic ecology derived from fatty acid and stable isotope composition. Thus, due to the increasing abundances reported (Buchholz et al. 2010), both the expatriate boreal euphausiids *M. norvegica* and *T. longicaudata* and the subtropical-boreal euphausiid *N. megalops* may be prospective species of increasing trophic relevance within the marine food-web of Kongsfjord. There has been a significant increase in the abundance of *M. norvegica* and *T. longicaudata* in the marginal ice zone of the Barents Sea (Dalpadado et al. 2008) and in the Kongsfjord in the last decades (Buchholz et al. in prep). The abundance of the most uncommon krill species appearing in the high Arctic fjord, the subtropical-boreal *N. megalops*, was found to have increased markedly within the seven year investigation period. In 2006, densities were as low as 0.1 individual per 100 m^3 (Buchholz et al. 2010). In late April 2013, Buchholz et al. (in prep.) repeatedly found a ten times higher abundance of up to 1.0 individual 100 m^{-3} (samples taken in Kongsfjord at 78.95°N 11.98°E). This number resembles the densities of the arcto-boreal *T. raschii* reported in earlier studies. Here, 1.3 individuals 100 m^{-3} were found by Weslawski et al. (2000) and 1.2 individuals 100 m^{-3} were reported by Buchholz et al. (2010). Nevertheless, to date, the highest abundances within the Kongsfjord ecosystem refer to the arcto-boreal *T. inermis*.

4.4.1 Total lipids – energetic value

From the lipid perspective, *T. inermis* is the only euphausiid investigated that showed all signs of a true polar zooplankton species: high mean lipid contents (> 40 % DW; range: 35 – 54 %), mainly stored in energy-rich wax esters (> 40 %; range 41 – 55 %) which are specially synthesized *de novo* from the exploitation of the spring and summer phytoplankton blooms (Falk-Petersen et al. 2000). Due to the high wax ester content, this species probably contains enough energy to survive the winter with a minimum of feeding (e.g. Falk-Petersen et al. 2000, Huenerlage et al. in rev.). There were no differences between males and females sampled in late August from both locations (Kongsfjorden and Hornsund), this probably resulted from the fact that the specimens were sexually regressed; i.e. neither spermatophores nor ovaries were visible and both sexual organs were morphologically reduced. Sexual regression was also reported in previous studies in *T. inermis* (Dalpadado and Ikeda 1989), *E. superba* (Kawaguchi et al. 2007) and in *M. norvegica* (Cuzin-Roudy and Buchholz 1999) and may serve as a functional adaptation to reduce metabolic costs over winter when the species are unable to sufficiently cover reproductive needs.

The high lipid content of *T. inermis* makes it one of the most important food sources for higher trophic levels. Obradovich et al. (2014) highlighted the role of *Thysanoessa* species in the recruitment and performance of economically important fish stocks being key prey within the Newfoundland-Labrador Shelf ecosystem (“the euphausiid hypothesis”). Furthermore, *T.*

inermis was reported to be a major and thus key food item in ringed seals (Lydersen 2014) and many ecosystem relevant fish species. Amongst others, they determine the recruitment success, individual growth and overall population condition of economically important North Atlantic fish species such as blue whiting (*Micromesistius poutassou*), herring (*Clupea harengus*), capelin (*Mallotus villosus*), haddock (*Melanogrammus aeglefinus)*, juvenile polar (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) [Gjøsæter et al. 2002, Dalpadado and Bogstad 2004, Dolgov et al. 2010, Dalpadado and Mowbray 2013].

In comparison to other krill species, e.g. *M. norvegica* (Spicer and Saborowski 2010), the lipids of *T. inermis* are accumulated in multiple lipid vesicles forming a saddle-like structure located in the species' cephalothorax (Buchholz et al. 2010). Up to 90 % of the total lipids can be stored in this 'lipid body' (unpublished, own observations). Its congeners *T. raschii* and *T. longicaudata* show the same morphological feature, accompanied by high amounts of triacylglycerols as storage lipids.

In our study, the total lipid content of the boreal *T. longicaudata* was similar to that of the arcto-boreal *T. inermis* (both > 40 % DW). In contrast, the lipid content of the arcto-boreal *T. raschii* and the boreal *M. norvegica* were significantly lower (~ 30 % of dry weight). Generally, *T. inermis* is known to co-occur with *T. raschii* (e.g. Einarsson 1945, Mauchline 1980, Harvey et al. 2012). Due to the differences in lipid content, Harvey et al. (2012) proposed *T. raschii* as a lower quality food item to potential predators than *T. inermis*. This may equally apply to the boreal *M. norvegica*. Therefore, with increasing diversity of these key species, potential predators will be exposed to food sources of different quality. On the other hand, the increased biomass of all the euphausiid species investigated, including the biomass of *T. inermis*, may be beneficial for higher trophic levels and therefore, may compensate for the lower nutritional quality of the single specimens (Dalpadado et al. 2012). Nevertheless, the very low total lipid content found in the species of subtropical-boreal origin has to be considered. The total lipid content of *N. megalops* (~ 10 % DW) was only 5 % above the lowest limit essential for membrane functioning, and therefore survival (Hagen et al. 2001). The contents were similar to those in the specimens from the Iceland Basin and somewhat less than in the specimens from the Benguela Current (on average 7.6 % DW). The values were comparable to those in the Mediterranean Sea samples (Cartes 2011), where the species shows low lipid levels from 7.3 to 11.3 % DW throughout the year. Therefore, the very low lipid content appears to be determined by its taxonomic affiliation as it is adapted to the specific environment with no or only short periods of food absence requiring only low or almost no lipid storage. Accordingly, despite the low abundances, *N. megalops* will be by far of the lowest nutritional quality to the Arctic predators in Kongsfjorden relying on lipid rich nutrition.

4.4.2 Lipid classes – energy storage strategy

Having a very low lipid content, *N. megalops* mainly biosynthesised polar lipids (> 70 %) consisting of ~ 26 % phosphatidylethanolamine and ~ 47 % phosphatidylcholine (~ 22 % and ~ 48 %, respectively, for specimens from Iceland Basin and Benguela Current). These are generally membrane lipids and are not used for energy storage purposes. Accordingly, if not continuously feeding, this species will probably not survive the polar winter when food sources are scarce. This is further confirmed by the low starvation capacity of 4 d found in this species (Boyd 1978).

In comparison, the major storage lipid of *T. raschii*, *T. longicaudata* and *M. norvegica* were triacylglycerols (47 %, 52 % and 68 % respectively). Triacylglycerols are generally known to serve as short-term energy storages which can be rapidly catabolised if energy is required (Lee et al. 2006). Therefore, they are primarily accumulated in zooplankton species from

lower latitudes with a more omnivorous or carnivorous feeding mode, which do not have to cope with long starvation periods as found in polar regions (e.g. Clarke 1980, Lee et al. 2006). There are, however, some polar species such as the endemic Antarctic krill species *Euphausia superba* (e.g. Meyer 2012), which also store high amounts of triacylglycerols. These species are unable to survive during polar winter without additional nutrition and overall metabolic reduction (loc. cit.). Accordingly, in order to survive the long period of food scarcity during Arctic polar winter, both the boreal species *M. norvegica*, *T. longicaudata* and the arcto-boreal species *T. raschii* will need additional energy input and/or other metabolism reducing adaptations similar to *E. superba*. As an example, the arcto-boreal *T. raschii* is believed to feed on sediments and detritus over winter to fulfil energy requirements. This was initially suggested from fatty acid analyses (Sargent and Falk-Petersen 1981) but also from stomach content analyses (Berkes 1976, Schmidt 2010). We often found *T. inermis* close to the sea bottom, particularly in extreme numbers at the end of winter in the Kongsfjord. Epibenthic and benthic feeding was also observed in *M. norvegica* (Greene et al. 1988, Schmidt 2010 and own observations). This is not yet known in the predominantly open ocean species *T. longicaudata*. It is reasonable that all three congeners may share this survival strategy. Additionally, being opportunistic omnivorous (Mauchline 1980), *M. norvegica* may feed on lipid rich (diapausing) copepods to overwinter. The same feeding behaviour was found in the Antarctic *E. superba* (Huntley et al. 1994) and may also be feasible for the mainly carnivorous *N. megalops* (see below).

4.4.3 Fatty acids and stable isotopes – feeding mode

Both the fatty acid trophic marker signals as well as the stable isotope studies suggested *N. megalops* as a predominantly carnivorous species occupying the highest trophic level among the species investigated. The relatively low percentage of wax esters found in *N. megalops* (1.8 – 11.7 % of total lipid) probably from dietary input of calanoid copepods containing high amounts of wax esters (Sargent and Falk-Petersen 1981). In all regions sampled, the major fatty acids were the 18:1(n-9) fatty acid as typical trophic marker for carnivory (Graeve et al. 1997) and the 20:1(n-9/n-7) and 22:1(n-11/n-9) fatty acids as markers for the ingestion of calanoid copepods (e.g. Legezynska et al. 2014). In contrast, diatom markers 16:1(n-7) and 18:1(n-7) (Stübing and Hagen 2003) only accounted for ~ 7 % of the total fatty acids. The relatively high PUFA/SFA and 18:1(n-9)/18:1(n-7) fatty acid ratios as well as the low 16:1(n-7)/16:0 fatty acid ratio further emphasized the carnivorous feeding habit (Auel et al. 2002). Due to the high content of polar lipids found in this species (see above), the remaining fatty acids, 22:6(n-3), 20:5(n-3) and 16:0, were mainly defined as constituents of membrane lipids rather than trophic indicators (Lee et al. 2006).

The preference for carnivory in *N. megalops* was also reported in other studies. It was either deduced from the species morphological appearance [lacking a feeding basket and having extended thoracic appendages most likely assisting predation (Mauchline et al. 1989)] and confirmed by gut content analyses (Gurney et al. 2001, Barange et al. 1991) or suggested from stable isotope analysis (Gurney et al. 2001) and analysis of fatty acid trophic markers (Mayzaud 2007, Cartes 2011).

Even higher percentages of fatty acids indicating a carnivorous feeding strategy were found in the boreal *M. norvegica*, although the stable isotopic signals of this species pointed to a generally omnivorous feeding habit [calculated trophic level of 2.6 = omnivorous (Søreide et al. 2006a)]. In comparison to the other four krill species sampled in Kongsfjorden, *M. norvegica* contained the highest amount of fatty acids originating from calanoid copepods (~ 30 % of the total fatty acids) pointing to opportunistic omnivorous and/or carnivorous feeding. This habit was also reported in other studies on *M. norvegica* sampled in North Atlantic

waters (e.g. Falk-Petersen et al. 2000, Kaartvedt et al. 2002, Petursdottir et al. 2008). Nevertheless, fatty alcohols were not present in the specimens sampled. This might be explained by the immediate catabolism of the *Calanus* wax esters shortly after ingestion and the subsequent incorporation into lipid storages.

Similar to *M. norvegica*, stable isotope analyses on the *Thysanoessa* species sampled in this study pointed to a generally omnivorous feeding (trophic levels 2.4 – 2.7 = omnivorous; Søreide et al. 2006a). Nevertheless, fatty acid trophic markers showed a higher tendency to more herbivorous feeding than in the previously discussed species (i.e. the subtropical-boreal *N. megalops* and the boreal *M. norvegica*). This was highlighted by multivariate correspondence analysis and is in accordance with other studies conducted on *Thysanoessa* spp. (e.g. Falk-Petersen et al. 1981). Some authors, however, described *T. raschii* and *T. longicaudata* to have a higher preference for carnivorous feeding than *T. inermis* (e.g. Sargent and Falk-Petersen 1981, Falk-Petersen et al. 1990, Falk-Petersen et al. 2000). At first sight, this does not resemble our results as interspecific differences in copepod markers were negligible between the three *Thysanoessa* species investigated. However, both *T. raschii* and *T. longicaudata* showed relatively high amounts of the monounsaturated fatty acid 18:1(n-9). The percentage was even higher compared to the rather carnivorous *M. norvegica* and *N. megalops*. 18:1(n-9) is a commonly used marker for carnivory, but it is also believed to be the major constituent in wax esters *de novo* synthesized by omnivorous zooplankton such as e.g. the arcto-boreal *Thysanoessa inermis* and the Antarctic *Euphausia crystallorophias* (Falk-Petersen et al. 2000). Wax ester levels were very low in *T. raschii* (~ 6 % of total lipid) and *T. longicaudata* (~ 3 % of total lipid) compared to *T. inermis* (up to 50 % of total lipids). Accordingly, the high amounts of the 18:1(n-9) fatty acid found in *T. raschii* and *T. longicaudata* may most probably result from carnivory on other metazoans (e.g. Falk-Petersen et al. 2000).

In summary, not only the nutritional value (see above) but also the feeding preferences of the different krill species may alter the ecosystem balance. Hence, increasing abundances will most likely result in stronger predation pressure on lower trophic levels (e.g. phytoplankton, copepods, fish eggs and fish larvae). From this follows a simultaneous increase in competition on shared food sources with other macro-and mesozooplankton. Especially the increased abundances of the predominantly carnivorous euphausiids *M. norvegica* and *N. megalops*, might lead to important interspecific competition with (juvenile) planktivorous fish. This has been reported for *M. norvegica* from other study areas (e.g. Beyer 1992, McBride et al. 2014). Even direct predation on fish larvae may occur.

4.5 Conclusion

It is not yet proven if the single krill species recently forming the euphausiid community of Kongsfjorden can thrive and persist at this high latitude. However, several future projections point to potential climatic shifts which might further improve conditions for the expatriate species of Atlantic origin (Falk-Petersen et al. 2007).

In our study, the five krill species clearly differed in their lipid biochemistry. The lipid contents of the species investigated ranged from > 40 % DW (*Thysanoessa inermis* and *T. longicaudata*) to lower than 30 % DW (*T. raschii* and *Meganyctiphanes norvegica*) to as low as ~ 10 % DW in *Nematoscelis megalops*. Further, the fatty acid profiles were either consistent with predominantly herbivorous feeding (arcto-boreal *T. inermis*), omnivorous feeding i.e. with higher tendency to carnivory and alternative food sources (arcto-boreal *T. raschii* and boreal *T. longicaudata*, *M. norvegica*) or mainly a carnivorous feeding habit (subtropical-boreal *N. megalops*).

Concerning the increased krill diversity in the high Arctic Kongsfjorden, we suggest that these interspecific differences may be of remarkable impact on ecosystem structure. Top predators relying on krill as a food source will be exposed to a new diet of most probably lower quality. Furthermore, the overall increase in the krill biomass may result in higher predation pressure on the lower trophic levels. In addition, due to the different feeding modes, increased competition on shared resources might be expected not only within the krill species but also with other macro- and mesozooplankton including planktivorous fish.

Considering the Arctic to be in a warming transition, knowledge on the population dynamics of the pelagic components in the Kongsfjord ecosystem is still scarce. In order to best predict possible future food-web implications, our results underline the need for detailed investigation of the single ecosystem components – both with respect to biochemical composition and population dynamics. The data collected in this study may therefore be useful as a basis for encompassing ecological modelling studies especially focussing on complex food-web dynamics.

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Operating the Tucker Trawl on-board the RV *Oceania* in Hornsundfjord 2012

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5 PUBLICATION IV

Thermal limits of krill species from the high Arctic Kongsfjord (Spitsbergen)*

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Abstract

The high Arctic Kongsfjord is influenced mainly by cold Arctic water but also warmer Atlantic water masses. In recent years, the proportion of the Atlantic inflow from the south has increased. Concurrently, one temperate-boreal (*Meganyctiphanes norvegica*) and one subtropical-temperate krill species (*Nematoscelis megalops*) are now regularly found in the Kongsfjord – in addition to the previously prevailing arcto-boreal species *Thysanoessa inermis* and *T. raschii*. In view of the recent changes in these species' biogeographic distributions, we compared their physiological tolerances. Using non-invasive optical oxygen sensors, respiration measurements served to characterize metabolic responses to temperature variations. *Thysanoessa* spp. appear more cold-stenotherm than the other two krill species: the upper level of respiratory capacity is reached at 12°C and they are less tolerant to decreasing oxygen concentrations. This finding is consistent with their arcto-boreal distribution. In contrast, *Meganyctiphanes norvegica* and *Nematoscelis megalops* showed a higher tolerance to temperature changes, a robust nutritional condition and sexual maturity. Such physiological plasticity may explain the recent northward expansion of their geographic range.

5.1 Introduction

Ambient temperature is a major factor influencing a species metabolic performance and hence, its survival and distribution (Pörtner et al. 2001, Pörtner 2002). Quantification of species-specific metabolic thermal limits is, therefore, helpful to predict acclimatization of species impacted by global warming (Somero 2005).

During the last decade, hydrographic studies have indicated a climatic shift within the Arctic, i.e. a transition to a warmer state (e.g. Polyakov et al. 2007, Spielhagen et al. 2011). This change has been attributed to an increased strength of the West Spitsbergen Current conveying warm Atlantic waters to the system (loc. cit). Concomitantly, boreal fauna spread to higher latitudes (Hop et al. 2002, Hop et al. 2006, Zhukova et al. 2009, Buchholz et al. 2010, Kwasniewski et al. 2012). The hydrography of the high Arctic Kongsfjord, located at the west coast of Spitsbergen (79°N), alternates seasonally between the dominance of the cold Arctic coastal waters (< 0°C) and the warm (≥ 4°C) Atlantic waters (e.g. Svendsen et al. 2002, Hop et al. 2002, 2006). Within this coastal regime, the krill community consists of *Thysanoessa inermis* and its congener *T. raschii* (e.g. Timofeyev 1993, Weslawski et al. 2000). Both species have an arcto-boreal distribution and are common in the Barents Sea, Norwegian Fjords as well as at lower latitudes, i.e. the Newfoundland and Labrador shelf (45-50°N, 47-55°W; e.g. Einarrson 1945, Mauchline & Fisher 1969, Timofeyev 1993, Buchholz et al. 2012). However, recently, the intrusion of the warm Atlantic waters into the Kongsfjord has increased the krill diversity. One species of temperate-boreal origin (*Meganyctiphanes norvegica*) and one species of subtropical-temperate origin (*Nematoscelis megalops*) are now

*) This manuscript is the latest pre-print version, which was accepted by Marine Ecology Progress Series. Accordingly, it is the improved version of that originally submitted in the PhD Thesis November 2014.

found regularly in the Kongsfjord and adjacent waters (e.g. Buchholz et al. 2010, Huenerlage et al. 2014). This condition provided an opportunity to address an important question: will the phenotypic plasticity of these species allow all of them to persist in high latitudes?
Oxygen consumption is a useful indicator of species-specific metabolism as it serves as a proxy for the sum of physiological processes (e.g. Williams & Del Giorgio 2005). Accordingly, to investigate the metabolic response to changing temperatures, temperature-controlled experiments were conducted to assess the respiration rates of each krill species. Further, fresh weight, stomach content, hepatopancreas colour, sexual development stage and excretion rates of each species were determined to assay overall health.

5.2 Material and methods

5.2.1 Sample collection

Adult krill were caught on-board the Kings Bay AS workboat MS Teisten (Kongsfjord at 79°N) using a 1 m² Tucker trawl (1000 µm mesh with soft cod-end bucket). The trawls were towed at a speed of two knots. Additionally, adult *Thysanoessa inermis* were collected in a similar manner from the Polish RV Oceania (Hornsund fjord at 77°N). Detailed information on the species' sampling locations is given in Fig. 5.1 and Table 5.1, respectively. The hauls were taken from 90 - 300 m depth depending on station and maximum abundance at the stations sampled (F. Buchholz pers. obs.).

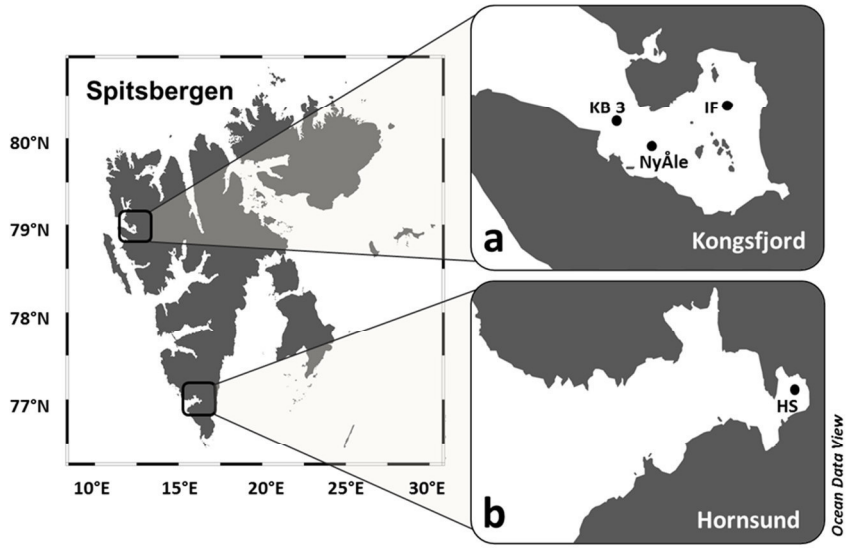

Fig. 5.1 *Map of Spitsbergen. Squares highlight the position of the Kongsfjord (a) and the Hornsund fjord (b). See also Table 5.1 for detailed station locations*

Immediately after collection, specimens were sorted by species and transferred to aerated aquaria filled with filtered seawater (0.2 µm) at *in situ* temperature (4°C) in dim light. After 12 h, the specimens were brought to experimental temperatures (i.e. 0, 2, 6, 8, 10, 12, 14 or 16°C) at a rate of 1°C h^{-1}, followed by 12 h acclimation at constant specific experimental temperature, before use in the respiration experiments. Hence, the total maintenance before measurement was not longer than 36 h (e.g. at 16°C).

Table 5.1 *List of krill species investigated with information on sampling period, station names and station positions (see also Fig. 5.1).*

			Species			
Name	Position		*Thysanoessa inermis*	*Thysanoessa raschii*	*Meganyctiphanes norvegica*	*Nematoscelis megalops*
HS	76.99°N	16.33°E	End of July 2012	–	–	–
KB3	78.96°N	11.97°E	April 2013	–	April 2013	April 2013
NyÅle	78.94°N	12.08°E	August 2013	August 2013	August 2012	August 2012
IF	78.97°N	12.36°E	August 2012 August 2013	August 2012 August 2013	–	–

5.2.2 Metabolic rate measurements

Acclimated specimens were individually incubated in closed tubular chambers (Perspex; 20 ml) specially designed for measuring routine metabolic rates in krill (Werner et al. 2012). The chambers were filled with (0.2 µm) filtered seawater at experimental temperature (i.e. 0, 2, 6, 8, 10, 12, 14 or 16°C) and stored in a water bath in the dark in a temperature-controlled refrigerator. Two chambers were prepared without a specimen and served as controls. The oxygen consumption (mg O_2 L^{-1}) was monitored every 30 sec by optode respirometry with a 10-channel optode respirometer (PreSens Precision Sensing Oxy-10 Mini, Regensburg, Germany). The measurements lasted 1.5 to 6 h, depending on the individuals' metabolic rates and were stopped at the latest when the oxygen concentration inside the test-chambers reached 60 % of the start concentration.

After the experiments, the specimens were scored under a stereomicroscope for individual sex, size (from the front of the eyes to the tip of the telson to the nearest mm), fresh weight in mg, stomach fullness, hepatopancreas colour (Morris et al. 1983) and sexual development stage (Cuzin-Roudy 1993). These parameters served as proxies for the species' overall health.

For the determination of ammonium (NH_4-N) excretion, water samples (500 µl) from each respiration chamber were frozen and stored at –80°C until measurement. The analysis was done in triplicates and conducted photometrically following the phenol-hypochlorite method according to Solorzano (1969) using a microplate reader (630 nm; Multiskan™ FC Microplate Photometer, Thermo Scientific, USA).

The atomic oxygen (O) to nitrogen (N) ratio was used as an indication for the substrate catabolized for energy provision: O:N < 24 for protein and O:N > 24 for lipid dominated metabolism (Mayzaud & Conover 1988).

5.2.3 Lowest tolerable ambient oxygen concentration in Thysanoessa inermis

Another experiment investigated the minimum tolerable oxygen concentration with increasing experimental temperature. Due to time limitations, the experiment was only conducted on *T. inermis* specimens. The experimental set-up was the same as for the metabolic rate measurements. However, the experiments were only conducted at 6 different temperatures (i.e. 2, 4, 6, 8, 10 and 16°C; n = 8 for each). Furthermore, this experiment used the optode

respirometer to monitor the oxygen concentration (mg L^{-1}) inside the chamber while the specimens' overall condition was monitored by eye. The measurements were stopped as soon as the specimens became immobile but the heart was still beating.

5.2.4 Data analysis

Metabolic rates were normalized to one mg fresh weight (FW) and expressed in µmol h^{-1}. Q_{10} values were calculated from respiration measurements according to van't Hoff (1884). The Arrhenius breakpoint temperatures (ABT) of the temperature-dependent oxygen consumption of *T. inermis* and *T. raschii* were estimated through two-phase regression conducted on the Arrhenius plots of the corresponding respiration rates (Dahlhoff et al. 1991; plots are given in the electronic supplement Fig. S1).

A one-way ANOVA with post-hoc Tukey test was used for general species comparisons. A one-way ANOVA with post-hoc Dunnett test was performed to test for the temperature influence on the specimens' respiration rates compared to 4°C control temperature. The same test was used to compare the temperature effect on the lowest tolerable oxygen concentrations of *T. inermis*. Species-specific seasonal differences (autumn vs. spring) were analysed with an unpaired t-test. Species-specific differences between genders were either analysed with an unpaired t-test (male vs. female) or one-way ANOVA (male vs. female vs. neuter).

All analyses were conducted using GraphPad Prism 5 (GraphPad Software, Inc., USA). The significance level was set at $p < 0.05$. All data are given as means ± standard error of the mean (SEM).

5.3 Results

In total, 437 euphausiids were sampled. Adult *Thysanoessa inermis* (total n = 282), *Meganyctiphanes norvegica* (total n = 85) and *Nematoscelis megalops* (total n = 10) were sampled both in early spring (first week of April 2013) and early autumn (August 2012/2013). *T. raschii* was only sampled in early autumn (n = 60; Table 5.1 and Table 5.2). In all parameters investigated, no intraspecific differences were found based on gender. Therefore the data were pooled for interspecies and seasonal comparisons, respectively (e.g. Table 5.2, Fig. 5.2 and Fig. 5.4).

5.3.1 Biometry and individual life parameters

During early autumn, the species differed significantly in mean total body length and mean total fresh weight ($p < 0.0001$, F = 53.4; $p < 0.0001$, F = 47.3). With a mean total length of 27.9 mm and a mean fresh weight of 166.2 mg, *M. norvegica* were the largest and heaviest individuals of the four krill species sampled, followed by *T. inermis* (24.8 mm, 110.3 mg), *T. raschii* (23.5 mm, 79.2 mg) and *N. megalops* (21.3 mm, 64.0 mg; Table 5.2). The difference was not significant between the species sampled in spring 2013. *T. inermis*, *M. norvegica* and *N. megalops* had the same body length (21.2 – 21.4 mm) and the same fresh weight (61.8 – 63.6 mg; Table 5.2).

The nutritional condition by measure of stomach fullness (STO) and hepatopancreas colour (HPP), was different between the species from both seasons ($p < 0.0001$, F = 12.7 in autumn; $p < 0.0001$, F = 16.6 in spring). Both in autumn and in spring, lowest stomach fullness was observed in *T. inermis* (STO ~ 0.4; see Table 5.2). Concomitantly, the hepatopancreas was colourless. The same was found in *T. raschii* in autumn (STO ~ 0.7, colourless HPP) and in

N. megalops in spring (STO ~ 0.4, colourless HPP). In autumn, highest stomach fullness was observed in *M. norvegica* and *N. megalops* (STO ~ 0.8, orange/white HPP and STO ~ 1.2, yellow HPP; Table 5.2). The difference was not significant. However, conspicuously, oil droplets were visible in some of the stomachs of the *M. norvegica* specimens. In spring 2013, *M. norvegica* had the highest stomach fullness (STO ~ 1.2) and was the only species showing a coloured HPP (yellow/green; Table 5.2).

Table 5.2 *Sex (percentaged composition; f = female, m = male, n = neuter), total length (L), fresh weight (FW), stomach fullness (STO; 0 – 2 = empty – full), hepatopancreas colour (HPP) and sexual developmental status (SDS) of adult krill species sampled for respiration measurements; n = number of individuals. See Table 5.1 for details of the sampling periods.*

		Parameter						
Species	Season	*n*	Sex (% f: m :n)	L (mm)	FW (mg)	STO	HPP	SDS
T. inermis	*Autumn*	201	46: 34 :20	24.8 ± 0.1	110.3 ± 1.9	0.4 ± 0.1	C	inactive
	Spring	81	23: 70 : 7	21.2 ± 0.2	61.8 ± 2.3	0.4 ± 0.1	C	active
T. raschii	*Autumn*	60	67: 33 : 0	23.5 ± 0.2	79.2 ± 2.4	0.7 ± 0.1	C	males active
	Spring	–	–	–	–	–	–	–
M. norvegica	*Autumn*	54	39: 44 :17	27.9 ± 0.5	166.2 ± 10.6	0.8 ± 0.1	O/W	males active
	Spring	31	43: 50: : 7	21.3 ± 0.8	61.8 ± 5.9	1.2 ± 0.1	Y/G	active
N. megalops	*Autumn*	7	29: 71 :0	21.3 ± 0.5	64.0 ± 4.6	1.2 ± 0.2	Y	active
	Spring	3	67: 33 :0	21.4 ± 0.4	63.6 ± 2.6	0.4 ± 0.1	C	active

Maturity was estimated by sexual developmental stage (SDS). *N. megalops* was the only species in which female and male specimens were sexually active in both seasons sampled (Table 5.2). The other species investigated were active only during spring. In autumn, only the male specimens of *T. raschii* and *M. norvegica* were mature, whereas all individuals of *T. inermis* were immature and/or sexually regressed (i.e. 20 % of all *T. inermis* specimens were defined as neuter; Table 5.2). Sexual regression was also found in the *M. norvegica* specimens (e.g. autumn: 17 % neuters, spring: 7 % neuters; Table 5.2).

5.3.2 Metabolic rates

Despite the seasonal differences found in biometric and individual life parameter investigations, respiration rates were the same in both seasons for all species sampled (See e.g. Table 5.1 for sampling details). Accordingly, for species comparison, the respiration rates were pooled for each experimental temperature (Fig. 5.2).

In *M. norvegica* and *N. megalops*, the increase in experimental temperature resulted in a significant (exponential) increase of oxygen consumption over the whole temperature range (0 - 16°C; *M. norvegica*: $p < 0.0001$, $F_{8,71} = 16.3$; *N. megalops*: $p < 0.0001$, $F_{2,7} = 56.6$; Fig. 5.2). Referred to 4°C control temperature, the increase was first significant at 12°C (Fig. 5.2; electronic supplement Table S1). The calculated Q_{10} values ranged from 1.5 to 2.4 in *M. norvegica* and from 1.6 to 2.0 in *N. megalops* (Table 5.3). A literature comparison of respiration-temperature curves of *M. norvegica* from different climatic zones is given in Fig.

5.3. It shows that the respiration rates of the specimens sampled in our study most closely resembled the values of those specimens sampled in the Clyde Sea. Nevertheless, the effect of temperature on the species' respiration rates showed the same pattern in all climatic zones (Fig. 5.3).

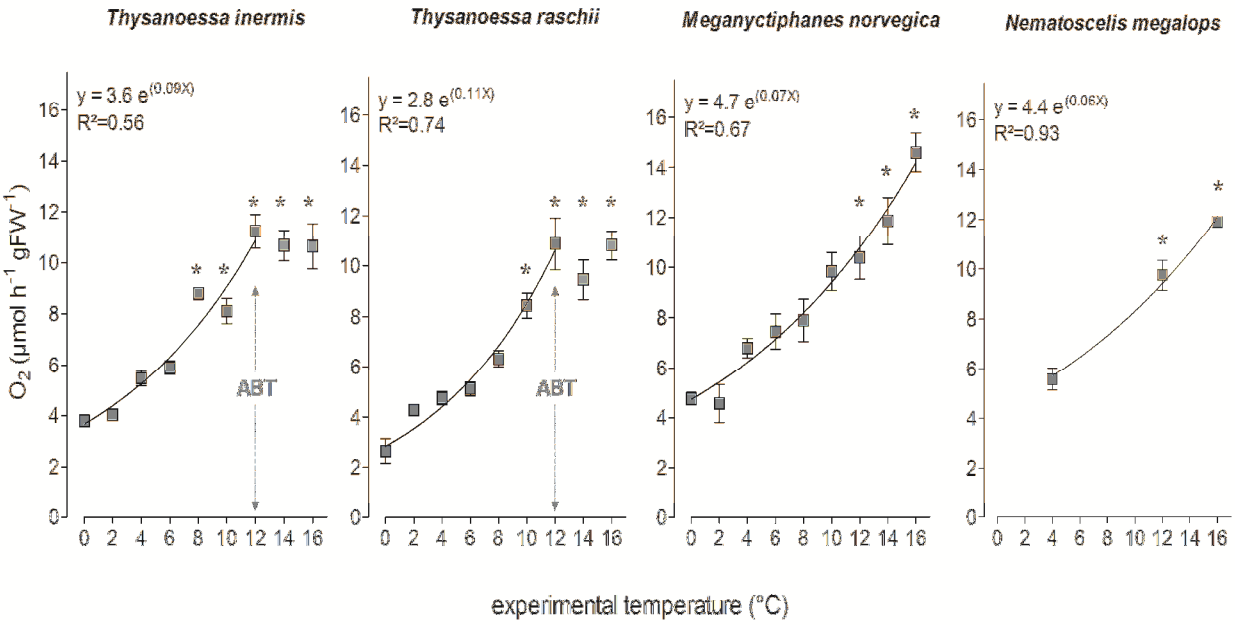

Fig. 5.2 *Mean (± SEM) respiration rates of adult Thysanoessa inermis (n = 20 – 35), T. raschii (n = 5 – 9), Meganyctiphanes norvegica (n = 5 – 19) and Nematoscelis megalops (n = 3 – 4) in relation to experimental temperature. ABT = Arrhenius breakpoint temperature. * Significant difference to 4°C control temperature (p < 0.05; one-way ANOVA with post-hoc Dunnett test)*

In contrast to *M. norvegica* and *N. megalops*, the species-specific respiratory performance during temperature increase in the two *Thysanoessa* species could be divided into two phases (Fig. 5.2; electronic supplement Fig. S1). Like in *M. norvegica* and *N. megalops*, in the first temperature increment (0 – 12°C), respiration rates increased exponentially. Referred to the 4°C control temperature, the increase was significant from 8°C in *T. inermis* and from 10°C for *T. raschii* (Fig. 5.2; electronic supplement Table S1). In the first phase, Q_{10} values ranged from 1.8 to 3.3 in *T. inermis* and from 2.3 to 3.9 in *T. raschii* (Table 5.3). However, at experimental temperatures beyond 12°C, mean oxygen consumption remained stable, i.e. fluctuated at ~ 11 µmol O_2 h^{-1} gFW^{-1}. Concomitantly, Arrhenius plots show a sharp change in the slope of linear regression indicating the respiratory Arrhenius breakpoint temperature (=ABT, Fig. 5.2; electronic supplement Fig. S1). The ABT was defined at 12°C in both species. Q_{10} values in the second phase were 1.0 for *T. inermis* and 0.9 for *T. raschii* (Table 5.3).

Table 5.3 *Q_{10} values of the mean respiration rates from adult Thysanoessa inermis (n = 20 – 35), T. raschii (n = 5 – 9), Meganyctiphanes norvegica (n = 5 – 19) and Nematoscelis megalops (n = 3 – 4); n = number of individuals used in the temperature experiments*

	Temperature range				
Species	0 – 4 °C	4 – 8 °C	4 – 12 °C	8 – 12 °C	12 – 16 °C
T. inermis	2.5	3.3	2.5	1.8	0.9
T. raschii	2.8	2.3	3.0	3.9	1.0
M. norvegica	2.4	1.5	1.7	2.0	2.3
N. megalops	–	–	2.0	–	1.6

In all species, ammonium excretion rates (NH_4-N) were positively related to the ambient temperature (Fig. 5.4). Seasonal differences were only found in the rates of *M. norvegica* (t-test e.g. at 8°C $p < 0.05$, df = 6; at 16°C $p < 0.05$, df = 7). Here, the mean excretion during spring was up to 3.5 times higher compared to the rates of specimens sampled during autumn (e.g. at 8°C and 16 °C; Fig.4; electronic supplement Table S1). In the other three species, mean excretion rates ranged from 0.3 to 1.8 µmol NH_4-N h^{-1} gFW^{-1} in *T. inermis* (0 – 16°C), from 0.8 to 2.3 µmol NH_4-N h^{-1} gFW^{-1} in *T. raschii* (0 – 16°C) and from 0.6 to 2.4 µmol NH_4-N h^{-1} gFW^{-1} in *N. megalops* (4 – 16°C; Fig.4; electronic supplement Table S1).

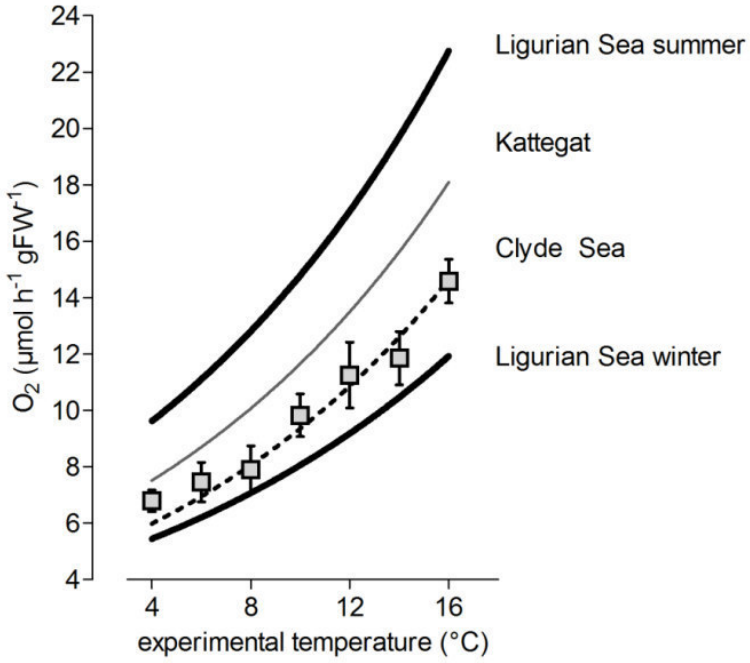

Fig. 5.3 *Comparison to literature. Temperature curve of mean (± SEM) respiration rates of adult Meganyctiphanes norvegica (squares; n = 5 – 19) in comparison to rates of M. norvegica from different climatic zones: Ligurian Sea summer, Kattegat (summer and winter), Clyde Sea (summer and winter) and Ligurian Sea winter (connecting lines; values converted from Saborowski et al. 2002 applying a dry to fresh weight conversion factor of 25 %, loc. cit.).*

The atomic oxygen to nitrogen (O:N) ratio was derived from the measurements of respiration and excretion. In both *Thysanoessa* species, this ratio was negatively related to increasing temperatures. For *T. inermis*, highest values were > 30 at 0°C and 2°C, respectively (Fig. 5.5; electronic supplement Table S1). The maximum O:N-ratio of *T. raschii* was ~ 24 at 4°C. Lowest values were calculated at temperatures above the ABT (O:N < 18 in *T. inermis* and O:N < 9 in *T. raschii*). In contrast, the O:N-ratios of *M. norvegica* and *N. megalops* were not influenced by experimental temperature. The ratio was < 24 for *N. megalops*. In *M. norvegica*, mean O:N-ratios were different between the sampling seasons. In autumn, mean O:N-ratios were > 24 with a minimum of 26.0 at 12°C and a maximum of 43.8 at 14°C, respectively (Fig. 5.5; see also electronic supplement Table S1). In comparison, spring ratios were < 24 and ranged from a maximum of 17.1 (4°C) to a minimum of 9.7 (16°C).

Table 5.3 *Q_{10} values of the mean respiration rates from adult Thysanoessa inermis (n = 20 – 35), T. raschii (n = 5 – 9), Meganyctiphanes norvegica (n = 5 – 19) and Nematoscelis megalops (n = 3 – 4); n = number of individuals used in the temperature experiments.*

	Temperature range				
Species	0 – 4 °C	4 – 8 °C	4 – 12 °C	8 – 12 °C	12 – 16 °C
T. inermis	2.5	3.3	2.5	1.8	0.9
T. raschii	2.8	2.3	3.0	3.9	1.0
M. norvegica	2.4	1.5	1.7	2.0	2.3
N. megalops	–	–	2.0	–	1.6

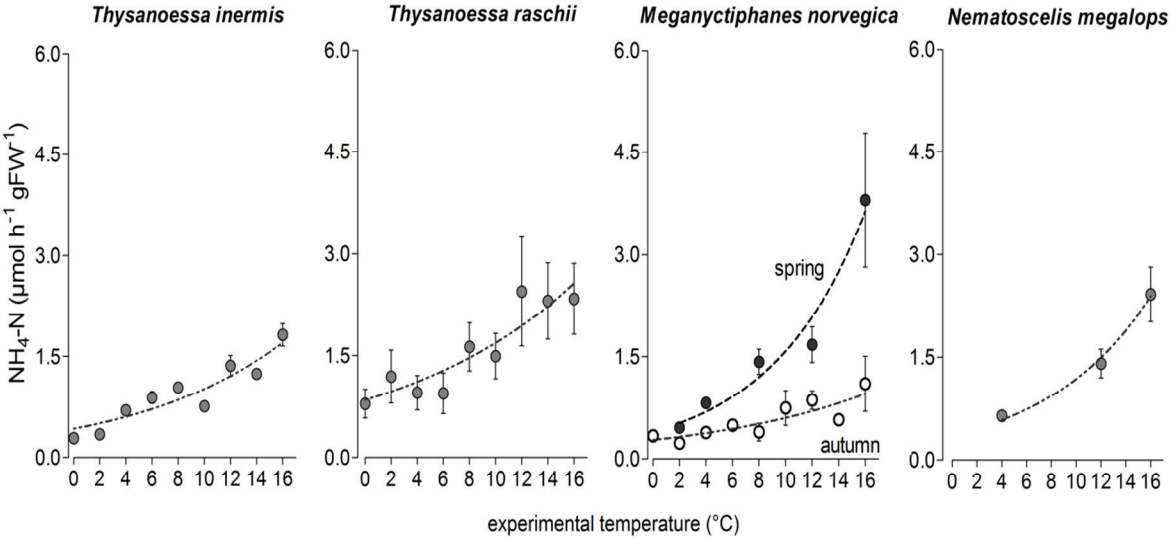

Fig. 5.4 *Mean (± SEM) excretion rates of adult Thysanoessa inermis (n = 20 – 35), T. raschii (n = 5 – 9), Meganyctiphanes norvegica (n = 5 – 19) and Nematoscelis megalops (n = 3 – 4) in relation to experimental temperature. For M. norvegica rates are shown for spring and autumn.*

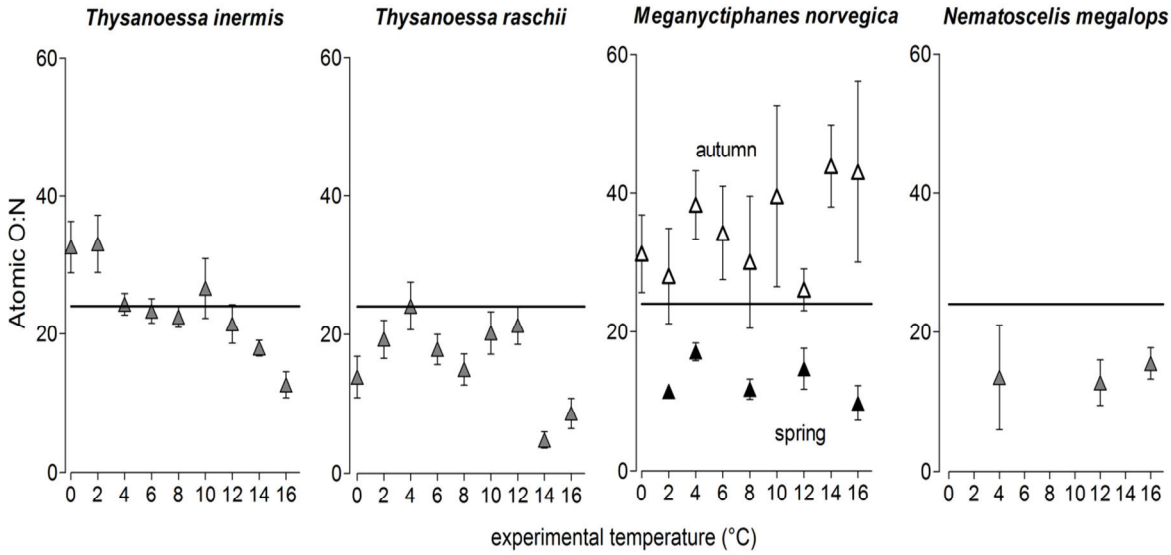

Fig. 5.5. *Mean (± SEM) oxygen to nitrogen (O:N) values of adult Thysanoessa inermis (n = 20 – 35), T. raschii (n = 5 – 9), Meganyctiphanes norvegica (n = 5 – 19) and Nematoscelis megalops (n = 3 – 4) in relation to experimental temperature. For M. norvegica rates are shown for spring and autumn. Solid line at O:N – ratio = 24 indicates equal use of proteins and lipids (Mayzaud & Conover 1988).*

5.3.4 Lowest tolerable ambient oxygen concentration in Thysanoessa inermis

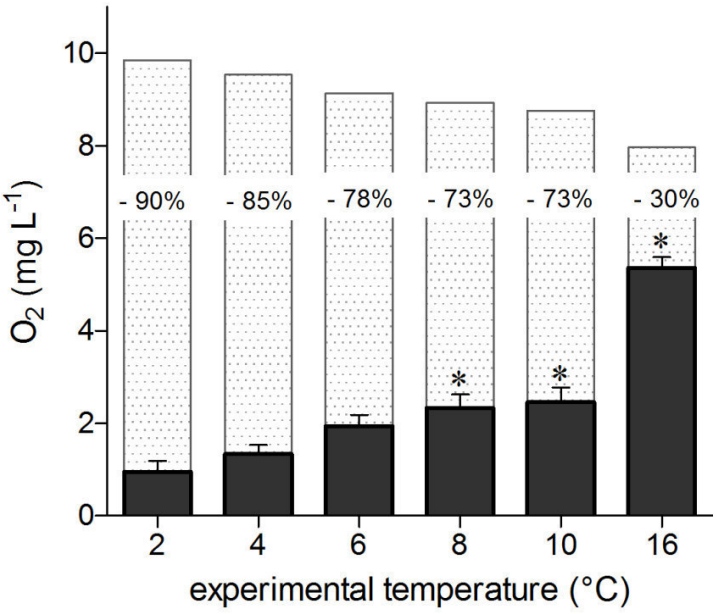

Fig. 5.6 *Minimum tolerable ambient oxygen concentration (dark bars = beginning of immobilization) of adult Thysanoessa inermis (n = 8 for each experimental temperature) in relation to experimental temperature. Percentages give the difference to the start concentrations (dotted bars = 100 % O_2 saturation at respective experimental temperature). Values are given as means ± SEM. * Significant difference to 4°C (p < 0.05; one-way ANOVA with post-hoc Dunnett test).*

The minimum tolerable oxygen (MTO) concentration for *T. inermis* was positively related to ambient experimental temperatures (Fig. 5.6). In comparison to the 4°C control group (MTO = 1.3 ± 0.2 mg L^{-1}), these values were significantly higher at temperatures > 8°C ($p < 0.0001$, F = 38.1). At 8°C, the average specimen did not endure (i.e. became physically immobile) ambient oxygen concentrations below 2.3 ± 0.2 mg L^{-1} (Fig. 5.6). The same was found for specimens incubated at 10°C (MTO = 2.4 ± 0.3 mg L^{-1}). At 16°C, *T. inermis* became physically immobile already at a mean ambient oxygen concentration of 5.3 ± 0.2 mg L^{-1} (= 70 % of start concentration; Fig. 5.6), which is only about one fourth the resistance compared to the control group (4°C) and/or even one sixth compared to 2°C (MTO = 0.9 ± 0.2 mg L^{-1}), respectively.

5.4 Discussion

5.4.1 The krill species' health

Our study showed that all specimens sampled (arcto-boreal *Thysanoessa inermis*, arcto-boreal *T. raschii*, temperate-boreal *Meganyctiphanes norvegica* and subtropical-temperate *Nematoscelis megalops*) were healthy. None of the species had empty stomachs, which indicated active feeding in both seasons sampled. However, the colour of the hepatopancreas (HPP) gave more detailed information on the species' nutritional condition. The HPP colour is an indicator for the species' long-term feeding activity, i.e. for the specific source of nutrition and can vary between colourless (no feeding), green (phytoplanktonic diet) and white, yellow, orange and red (zooplanktonic diet; e.g. Morris et al. 1983, Buchholz et al. 1989, Meyer et al. 2010). Accordingly, despite the stomach fullness, the colourless HPP in the *Thysanoessa* species and *N. megalops* suggested only occasional feeding. In contrast, the coloured HPP of *N. megalops* during autumn and *M. norvegica* during both seasons indicated a considerable fraction of zooplankton in these species' diet and hence, highlights their carnivorous tendency (e.g. feeding on lipid-rich calanoid copepods; Falk-Petersen et al. 2009, Huenerlage et al. 2014). The assumption of carnivory in *M. norvegica* was further supported by oil droplets present in some of the stomachs of those specimens sampled during autumn, which most likely originated from copepod ingestion.

M. norvegica was the only species that showed seasonal differences in excretion rates. The highest excretion rates occurred in the early spring samples. Excretion closely relates to the food ingested, i.e. high phytoplankton concentrations correspond to high ammonium excretion rates (Saborowski et al. 2002). Therefore, we suggest that *M. norvegica* shows more intensive feeding compared to the *Thysanoessa* species and *N. megalops* and hence, it is most likely the first krill species, which utilized the beginning spring phytoplankton bloom.

Sexual maturity was monitored as a first indicator of each species' reproductive potential. Our investigations showed that all specimens sampled were sexually active during spring 2013. Due to time-limitations, it was not possible to follow the onset and duration of species' mating or spawning periods. Other recent studies (e.g. Niehoff et al. 2013, F. Buchholz at al. 2010 and unpubl. data) have detected juvenile *Thysanoessa* spp. and *M. norvegica* from sediment traps and net catches in springtime in the wider Kongsfjord area, indicating successful spawning. Juvenile stages of *N. megalops* have not been detected in this region, even though this species was sexually active at the end of the summer. The other three species exhibited sexual regression, which is a typical functional adaptation to reduce metabolic costs when species are unable to sufficiently cover reproductive needs, for example during overwintering (e.g. Dalpadado & Ikeda 1989, Cuzin-Roudy & Buchholz 1999, Huenerlage et al. 2015).

5.4.2 Temperature effects on respiration rates

The experiments revealed that both the temperate-boreal *M. norvegica* and the subtropical-temperate *N. megalops* were respiratorily able to compensate for experimental changes in ambient water temperatures and associated changes in energy (oxygen) demands. Respiration rates were positively correlated to temperature and showed a characteristic Q_{10} relationship over the whole temperature range investigated (e.g. Q_{10} 1.6 – 2.7 computed for marine zooplankton including krill; in Ikeda 1985, Ivleva 1980). Remarkably, the metabolic reactions to temperature were the same compared to previous investigations performed at lower latitudes. In *M. norvegica*, our Kongsfjord-data (79°N) were comparable to populations sampled in the Kattegat (57°N; summer and winter) and the Ligurian Sea (43°N; summer), and correlated best to specimens sampled from the Clyde Sea (56°N; summer and winter; Saborowski et al. 2002). In *N. megalops*, temperature dependent respiration rates were similar over the entire temperature range (4 – 20°C) compared to specimens sampled from the subtropical-tropical Northern Benguela Current located at the SE-Atlantic at ~ 23°S (Werner et al. 2012). The comparison between the climatic zones indicated that temperature acclimatization (see Clarke 1991 for definition) did not seem to play a significant role within both krill species. Furthermore, at least between 0 – 16°C (*M. norvegica*) and 4 – 20°C (*N. megalops*), the specimens have not yet reached their thermal limits in all regions sampled, and experimentally were not yet exposed to their specific upper or lower respiratory pejus temperatures (see below). These findings highlight the considerable metabolic plasticity of these species and concurrently, support observations of their increased abundance at higher latitudes (e.g. Zhukova et al. 2009). However, it remains unclear whether the subtropical-temperate species is adapted to temperatures < 4°C. In comparison to the temperate-boreal *M. norvegica*, a low pejus temperature between 0 and 4°C in *N. megalops* may affect its survival, which may implicate population failure during transport northward.

In comparison to *M. norvegica* and *N. megalops*, the metabolic response to increasing temperatures in the two *Thysanoessa* species (*T. inermis* and *T. raschii*) was remarkably different, especially at the upper temperature ranges. In these species, an acute increase of experimental temperatures resulted in considerable respiratory disturbance. The Arrhenius breakpoint temperature (ABT) was defined at ~ 12°C after which respiration rates levelled out and became independent of further temperature increase, accompanied by a higher sensitivity to decreasing oxygen concentrations inside the respiration chambers (this study) and increased specimens' mortality during acclimation (K. Huenerlage et al. unpubl. data). According to Frederich & Pörtner (2000), this turning point most likely indicated the beginning of pejus hemolymph oxygenation and can therefore be equated to these species' upper pejus temperature limit (TpII). The effect of pejus temperature was further observed by a decrease of the Q_{10} values calculated at temperatures > 12°C (Pörtner & Knust 2006). Consequently, the ABT characterized the upper limit of temperature-induced oxygen demand in both *Thysanoessa* species and accordingly, their limit of metabolic (cardiovascular) capacity, likely followed by the onset of anaerobic metabolism (sensu Sokolova & Pörtner 2003).

However, metabolic response to short-term induced temperature changes may only reflect an individual's passive survival strategy and hence, time-limited tolerance. The specific thermal tolerance of an organism results from the complex interaction of small scale constituents (e.g. molecules, organelles, cells and tissues) that determine the mechanisms responsible for long-term survival, including the success of individual growth and reproduction and thus, overall, population performance and persistence (Pörtner 2002, Somero 2010). It is therefore interesting that after longer exposure to only 10°C, *T. inermis* specimens did not reduce their metabolic rates (due to possible further acclimation effects; K. Huenerlage et al. unpubl. data) and morphologically exhibited a red colouring of the exoskeleton, known as an indication for physiological stress (Buchholz 2003, Auerswald et al. 2008). Furthermore, investigations on

the molecular repair mechanisms of *T. inermis* [the heat-induced gene expression of the molecular chaperone 'heat shock protein 70' (Hsp70)], resulted in significant expression already at 10°C experimental temperature (personal communication Dr. K. Cascella, Station Biologique de Roscoff, France; K. Huenerlage et al. unpubl. data). Consequently, especially by considering long-term changes in temperature, the thermal limit found for the *Thysanoessa* species may even be below 12°C.

As a result, reports on these species' decreasing abundances at lower latitudes (e.g. in the Behring Sea and in the Newfoundland and Labrador shelf ecosystem; Colbourne et al. 2013, Coyle et al. 2011, Hunt et al. 2011, Coyle et al. 2008) may be related to their metabolic thermal limit. In the Newfoundland and Labrador shelf ecosystem, for instance, persistent warming anomalies sometimes exceeded the upper metabolic pejus temperatures suggested for the *Thysanoessa* species (summer surface temperatures of up to 15°C; see Colbourne et al. 2013). Consistent warming may have led to a loss of metabolic performance in both species and hence, may have altered the long-term population persistence of *T. inermis* and *T. raschii* within this region or at lower latitudes, respectively. Alternatively, our findings may highlight these species' strict arcto-boreal biogeographic distribution associated to colder water temperatures.

To our knowledge, respiratory upper thermal limits have not yet been detected in any other euphausiid species. At lower temperature ranges, respiration curves of both *T. inermis* and *T. raschii* are available from the literature (e.g. Sameoto 1976, Ikeda & Skjoldal 1989, Agersted et al. 2011) and are consistent with the species-specific respiration rates measured in this study. However, none of these investigations were conducted at temperatures at which the specimens may have exceeded their thermal respiratory capacity. This type of experiment is needed (especially with regard to species-specific thermal limits) to predict the persistence and possible establishment of populations in a given environment (Pörtner & Farrell 2008).

5.5 Conclusion

Our study on the upper thermal tolerances of krill from the Arctic offers an explanation of why both arcto-boreal *Thysanoessa inermis* and *T. raschii* were less numerous during warm periods at lower latitudes (e.g. Hunt et al. 2011, Coyle et al. 2008). Furthermore, this study highlighted the metabolic plasticity of the temperate-boreal *Meganyctiphanes norvegica* and subtropical-temperate *Nematoscelis megalops*, which supports the presence of higher stocks at lower latitudes as well as in a high Arctic fjord. The temperature-induced respiration curves indicate that both species are adapted to wide range of temperature fluctuations. However, low pejus temperatures may occur in *N. megalops*, which may constrain its persistence at high latitudes. In conclusion, the metabolic plasticity of *M. norvegica* may allow recruitment in "Atlantification" of the high Arctic Kongsfjord ecosystem, whereas *Thysanoessa* species are unlikely to acclimate when water temperatures warm.

5.6 Acknowledgements

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Towing for krill in the high Arctic Kongsfjord on-board MS *Teisten*

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6 PUBLICATION V

Towards finding the upper thermal limit of the arcto-boreal krill *Thysanoessa inermis*: Linking physiology to transcriptomics – first results

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Publication in preparation for submission

Abstract

Recent studies indicated a metabolic temperature sensitivity in both the arcto-boreal krill species *Thysanoessa inermis* and *T. raschii* which may determine these species' abundance and population persistence at lower latitudes. *T. inermis* currently dominates the krill community in the Barents Sea and in the high Arctic Kongsfjord. We aimed to increase the knowledge on the upper thermal limit found in this latter species (at ~ 12°C) by linking metabolic rate measurements with molecular approaches. Optical oxygen sensors were used to measure respiration rates in steps of 2°C (from 0°C to 16°C). To follow temperature-mediated mechanisms of passive response, i.e. as a proxy for molecular stress, we monitored the gene expression of the molecular chaperone heat shock protein 70 (Hsp70) during a sudden temperature exposure to 10°C with subsequent recovery at 4°C. Our results confirmed the presence of three (out of five) inducible *Hsp70* isoforms. The high level of gene expression during recovery (up to 80-fold) indicated that the specimens have experienced molecular damages during temperature exposure. These findings confirmed the temperature sensitivity of *T. inermis* and suggest the upper thermal limit even below 12°C. The study gives a baseline and a basis for further studies concerning the thermal limit of arcto-boreal *Thysanoessa* spp. and in comparison to other krill species under different climatic regimes.

6.1 Introduction

The arcto-boreal krill *Thysanoessa inermis* is well adapted to the Arctic environment mainly determined by low (water) temperatures, strong seasonality in light conditions and hence, in primary production (Hop et al. 2006, Buchholz et al. 2012). As a well-established expatriate from the Barents Sea, it currently dominates the krill community in W-Spitsbergen fjords including the high Arctic Kongsfjord at 79°N (e.g. Buchholz et al. 2010).

The Kongsfjord is mainly influenced by two different water masses which determine abiotic conditions (e.g. temperature, salinity, nutrients) within this ecosystem: the cold Arctic current and the warm West Spitsbergen Current. The West Spitsbergen Current is the major transport of heat from the Atlantic to the Arctic and particularly intruding the fjord during Arctic summer (Svendsen 2002, Hop et al. 2006).

During the last decade, continuous hydrographic studies pointed to a climatic shift within the Arctic, i.e. to a transition to a warmer state attributed to a stronger influence of warm Atlantic water masses (e.g. Polyakov et al. 2007, Spielhagen et al. 2011). For the ecosystem of Kongsfjord, this was especially measurable during the last three years. Here, the inner part of Kongsfjord remained ice-free over winter (e.g. in 2011/2012, 2012/2013 and 2013/2014; personal communication AWIPEV station leader Rudolf Denkmann) documented by mean

sea surface temperatures > 0°C from October to January (e.g. for 2012/2013 and 2013/2014 see COSYNA ferrybox at Spitzbergen operated by AWI and HZG Geesthacht; http://codm.hzg.de/codm). Furthermore, exceptional warm water temperatures of up to 8°C were recorded during summer 2014 (July/August; ref. COSYNA ferrybox) which were two degrees higher compared to the mean temperatures summers in 2012 and 2013 but almost double compared to maximum values reported a decade ago (3 – 4°C; Svendsen et al. 2002).

Remarkably, the population persistence at lower latitudes, i.e. abundance of especially the *Thysanoessa* species, *T. inermis* and *T. raschii*, was found to be determined by ambient water temperatures (e.g. Coyle et al. 2011, Hunt et al. 2011). Furthermore, a recent study of Huenerlage and Buchholz (submitted) highlighted the thermal metabolic sensitivity of both the *Thysanoessa* species as a potential explanation for the species' fate in biogeographic distribution. On the whole animal level, the authors found that the species were not able to respiratorily compensate the metabolic oxygen demands for ambient temperatures exceeding 12°C; i.e. the species were not able to maintain cardiac activity at temperatures ≥ 12°C and hence, suffered oxygen limitation due to limited capacity for oxygen uptake and/or oxygen transport mechanisms. Nevertheless, the authors mentioned the need for further investigations on small scale constituents like e.g. the induction of molecular chaperones to estimate or even verify the thermal limit of these species or, at least, to better understand and predict the survival of these species. Accordingly, questions arise about whether the specimens are merely "passively tolerating" increased temperatures or whether the thermal limit of long-term survival may be already reached at temperatures < 12°C.

If the species' long-term thermal limit would be below 12°C, then the current summer water temperatures of up to 8°C (see above) may already come close to the true thermal limit of the arcto-boreal *Thysanoessa* species. Hence, in the future, these species may experience maximum water temperatures close to their thermal limit, which may negatively affect their overall metabolic performance and thus regional persistence.

During a sudden temperature exposure to 10°C, we aimed to follow the molecular repair mechanisms of *T. inermis*, i.e. the temperature-mediated mechanisms of passive response. As a first indication, the heat-induced gene expression of the molecular chaperone heat shock protein 70 (Hsp70) may help to understand the thermal adaptive (i.e. survival) capacity of *T. inermis* within the changing ecosystem of the high Arctic Kongsfjord. The study will increase the knowledge on the adaptive potential of this species regarding its physiological reaction to sudden temperature exposure and accordingly, from the molecular point of view, consider the validity of the thermal limit found by Huenerlage and Buchholz (submitted). Furthermore, the present study may be of benefit for ecophysiological approaches in order to add more precise predictions on the metabolic performance of krill within in the changing ecosystem of the high Arctic Kongsfjord.

6.2 Material and methods

6.2.1 Sample collection

Krill (*Thysanoessa inermis*) were sampled in late summer 2012 (August 17th – 28th) on-board the Kings Bay AS workboat MS *Teisten* in the inner part of the high Arctic Kongsfjord (W-Spitsbergen) at 78.95°N, 12.33°E. A 1m² Tucker trawl (1000 µm mesh size and soft cod-end bucket) at a speed of two knots was deployed.

Immediately after catch, adult *T. inermis* were transferred to aerated aquaria containing filtered seawater and kept at 4°C in dim light before use in the experiments (respiration measurements and heat shock experiment, see below).

6.2.3 Respiration measurements

After 12 h, *T. inermis* specimens were randomly chosen for the respiration measurements. In groups of 10 to 20 individuals, the specimens were brought to experimental temperatures at a rate of 1°C h^{-1} (i.e. to 0, 2, 6, 8, 10, 12, 14 or 16°C), followed by 12 h acclimation at constant specific experimental temperature. Hence, to avoid starvation effects on the metabolic rates, the total maintenance before measurement was not longer than 36 h (e.g. at 16°C).

After acclimation, the specimens were individually incubated in closed tubular respiration chambers (Perspex; 20 ml) specially designed for measuring routine rates in krill (e.g. Werner et al. 2012, Huenerlage and Buchholz 2013). The chambers were filled with filtered seawater at experimental temperature and stored in a water bath in a temperature controlled refrigerator. The oxygen consumption (mg O_2 L^{-1}) was monitored every 30 seconds using a 10-channel optode respirometer (Oxy-10 Mini; PreSens Precision Sensing, Germany). The set-up allowed to measure up to 8 individuals in parallel. Two chambers were prepared without a specimen and served as controls. After the experiments, the specimens were weighed (mg) and scored for individual size (from the front of the eyes to the tip of the telson to the nearest mm).

6.2.4 Heat shock experiment

After 24 h, *T. inermis* specimens were randomly chosen for the heat shock experiment. In contrast to the respiration measurements, which used animals from the whole sampling period, the heat shock experiment only used specimens sampled on August the 17th, 2012.

The experiment was started by immediately transferring ~ 200 specimens from the lab maintenance at 4°C to one aquarium (30 L) containing aerated seawater at 10°C ('heat shock'). After 3 hours, one set of specimens (n = 50) were re-placed to the control temperature at 4°C for recovery. The recovery time lasted 6 hours. The same procedure was repeated with the remaining specimens 6 hours after heat shock. Both during heat shock and recovery time, subsamples of 10 specimens were taken every one and a half hour. The individuals were shock-frozen in liquid nitrogen and stored at -80°C until further analysis at the Station Biologique de Roscoff, France.

In parallel to the experiment, one group of *T. inermis* was kept at 4°C and served as control, i.e. was subsampled synchronously with the specimens from the heat shock experiment.

6.2.5 Characterisation of Hsp70 isoforms and cDNA cloning

To confirm the Illumina gene assemblies and the Hsp70 contigs, nested PCR and sequencing were performed before following *Hsp70* expression kinetics (Cascella et al. in prep.).

Ribonucleic acid (RNA) was isolated from the abdominal muscle of individual *T. inermis* specimens following the RNeasy® protocol (ref. Qiagen N.V., Netherlands). Concentrations of total RNA were determined photometrically at 260 nm using a Nanodrop® (Thermo Fisher Scientific, USA). RNA purity was reviewed by the A260/A280 ratio (i.e. absorbance at 260 nm to the absorbance at 280 nm).

Purified RNA (1 µg) was retrotranscripted into single stranded complementary deoxyribonucleic acid (cDNA) using SKdT primers (Roche, France) and the M-MLV Reverse

Transcriptase kit (Affymetrix USB®, USA) according to manufacturer's instructions. The *Hsp70* isoforms were PCR amplified from the cDNA using specific primers that were designed on sequences obtained from the Illumina assembly (Cascella et al. in prep.). Four pairs of PCR primers were used for each gene in order to clone each isoform in overlapping 1000 base pair sections to facilitate full depth sequencing of the whole gene. PCR products were gel purified and amplicons were inserted into the pGEM®-T Vector (Promega Corporation, USA). Plasmids were transformed into DH5α bacteria (*Escherichia coli*; Life Technologies™, USA). Transformed bacteria were selected and positive clones were verified by PCR. Plasmids were then extracted and sequenced with same primers as before.

6.2.6 Hsp70: quantitative polymerase chain reaction (qPCR)

Messenger RNA (mRNA) levels of the *Hsp70* isoforms were determined by reverse transcription qPCR amplification. Reactions were performed in 5 µl total volume containing 2.1 µl of diluted reverse transcription product (1:200), 0.4 µmol of each specific primer and 2.5 µl of SYBR Green I master mix (Roche, France). The amplification was carried out at 95°C for 15 min, then in 55 cycles at 95°C for 10 sec and at 60°C for 30 sec. A dissociation curve was generated and PCR efficiency was estimated for each primer pair. All primer pairs tested generated a single peak in the dissociation curve and a PCR efficiency of 80 - 100%. Data were analysed with the LightCycler 480 software (Roche, France). The 18S gene was chosen as a reference gene using the BestKeeper algorithm (Pfaffl et al. 2004) after testing EF1α, 18S, RPL8, and GAPDH as potential normalising housekeeping gene. *Hsp70* expression was subsequently normalized to this reference.

6.2.7 Data analysis

Metabolic rates were normalized to one mg fresh weight (FW) and expressed in µmol per hour (h^{-1}). The Arrhenius break temperatures (ABT) of the temperature dependent respiration curve was estimated from the Arrhenius plots of the corresponding respiration rates (Dahlhoff et al. 1991). A one-way ANOVA with post-hoc Dunnett test was performed to test for the temperature influence on the specimens' respiration rates.

Differences of the mean normalized expression (MNE) of the five *Hsp70* isoform genes (B, C1, C2, D and E) over time of the experiment were analysed using a non-parametric Kruskall–Wallis test. Relative gene expression (fold *Hsp70* expression) was calculated from the MNE of test specimens (heat shock at 10°C for 3 or 6 hours and subsequent recovery at 4°C) divided by the MNE of control specimens (kept at 4°C control temperature).

Statistical analyses were done used GraphPad Prism 5 (GraphPad Software, Inc., USA). The significance level was set at $p < 0.05$. Unless stated differently, data are given as means ± standard error of the mean (SEM).

6.3 Results

6.3.1 Metabolic rates

In total, 122 adult *Thysanoessa inermis* were sampled for the respiration measurements. Of these, 46 % were determined as females, 38 % as males and 16 % were determined as neuter due to sexual regression which did not allow for a clear sex determination. The specimens had an average fresh weight of 115.74 ± 4.2 mg and an average size of 24.5 ± 0.2 mm. There was no significant size difference between the sexes. Furthermore, the respiration rates did not differ between sexes. Therefore the data were pooled for the comparison of experimental temperatures (Table 6.1, Fig. 6.1).

Table 6.1 *Mean respiration rates of adult Thysanoessa inermis (n = 4 – 40) in relation to experimental temperature. Values are given as means ± SEM, n = number of individuals used in the temperature experiments. * Significant difference to 4°C control temperature.*

	Experimental temperature (°C)								
	0	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
n	8	8	32	8	8	40	6	8	4
Respiration rate ± SEM (μmol O_2 h^{-1} gFW^{-1})	3.3 ± 0.4	3.8 ± 0.4	4.7 ± 0.2	6.3 ± 0.5	7.8* ± 0.3	8.6* ± 0.4	10.2* ± 0.9	9.5* ± 0.9	7.3 ± 2.1

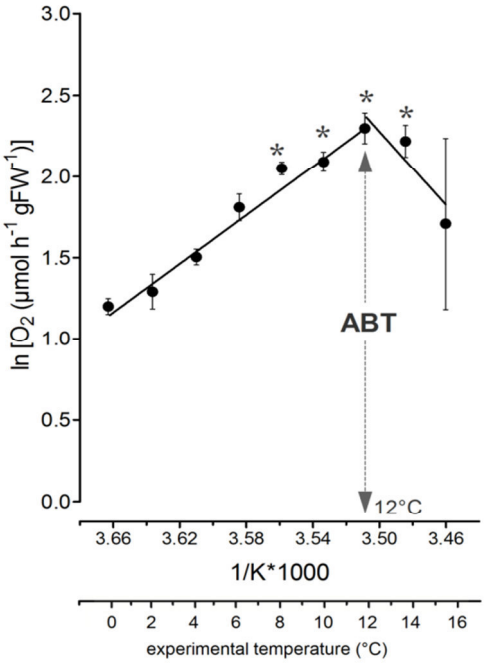

Fig. 6.1 *Arrhenius plot of normalized respiration rates of adult Thysanoessa inermis (n = 4 - 40) in relation to experimental temperature. Lines show linear regressions. ABT = Arrhenius breakpoint temperature defined by a significant change in slope. Values are given as means ± SEM, n = number of individuals used in the temperature experiments. * Significant difference to 4°C control temperature.*

The normalized respiratory performance over experimental temperature could be divided into two phases (Fig. 6.1). In the first temperature increment (0 – 12°C), respiration rates increased exponentially. Referred to the 4°C control temperature, the increase was significant from 8°C (4.7 ± 0.2 µmol O_2 h^{-1} gFW^{-1} at 4°C vs. 7.8 ± 0.3 µmol O_2 h^{-1} gFW^{-1} at 8°C; Table 6.1, Fig.1; $p < 0.0001$, F = 15.2, one-way ANOVA with Dunnett's Multiple Comparison Test against 4°C control temperature). However, at experimental temperatures beyond 12°C, mean oxygen consumption decreased from 10.2 ± 0.9 µmol O_2 h^{-1} gFW^{-1} at 12°C over 9.5 ± 0.9 µmol O_2 h^{-1} gFW^{-1} at 14°C to 7.3 ± 2.1 µmol O_2 h^{-1} gFW^{-1} at 16°C. The turning point was depicted by the Arrhenius plot and showed the respiratory Arrhenius breakpoint temperature at 12°C indicated by a sharp change in the slope of linear regression (=ABT; Fig. 6.1).

6.3.2 Hsp70 gene expression

During the whole 'heat-shock experiment' (= heat-shock and recovery after 3 and 6 hours exposure to 10°C), the gene expression of both the *Hsp70*-B and the *Hsp70*-E isoforms in the muscle tissue of *T. inermis* remained stable i.e. fluctuated between under-expression (2-fold decrease) to 4-fold increase relative to the control. In contrast, the other isoforms (*Hsp70*-C1, *Hsp70*-C2, and *Hsp70*-D) were significantly up-regulated, both under heat-shock conditions as well as during recovery ($p < 0.0001$; Fig. 6.2).

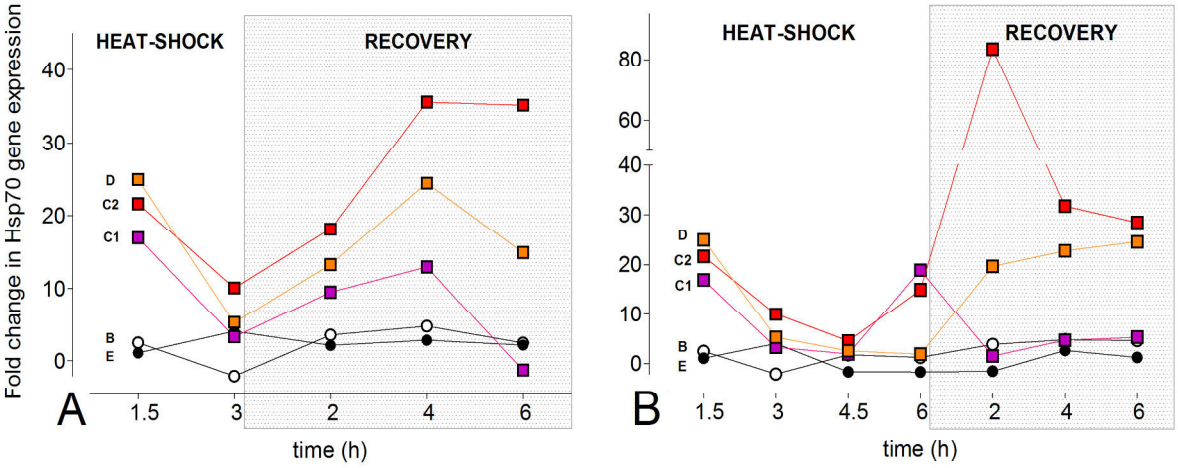

Fig. 6.2 *Mean Hsp70 ("Heat shock protein 70") expression ratio in muscle tissue of adult Thysanoessa inermis (n = 6 – 10) during 3 h (A) and 6 h (B) continuous heat shock at 10°C followed by 6 h of acclimation at 4°C control temperature (= recovery; shaded). Values relate to the control group (specimens continuously kept at 4°C control temperature). Letters belong to the adjacent symbols and indicate specific isoforms of the Hsp70.*

Under heat-shock conditions at 10°C (Fig. 6.2), all the three isoforms followed a similar pattern, i.e. the mRNA level increased significantly after the first 1.5 hours of heat-exposure (compared to the control: 17-fold in *Hsp70*-C1, 22-fold in *Hsp70*-C2 and 25-fold in *Hsp70*-D; Fig. 6.2) and decreased gradually until 4.5 hours after the start of the experiment (compared to the control: 2-fold in *Hsp70*-C1, 5-fold in *Hsp70*-C2 and 3-fold in *Hsp70*-D, Fig. 6.2B). Specimens exposed for 6 hours to 10°C experimental temperature showed a second increase in transcript levels in the *Hsp70*-C1 and *Hsp70*-C2 isoforms at the end of the heat exposure (19-fold in *Hsp70*-C1, 15-fold in *Hsp70*-C2).

During recovery, strongest expression was found in the *Hsp70*-C2 gene. After 6 hours heat exposure, the transcript level was up-regulated 80-fold after only 2 hours recovery. In the same isoform, 3 hours exposure resulted in a 35-fold increase of gene expression after 4 and 6 hours recovery. *Hsp70*-C1 was the only isoform that did not show a further increase in gene expression during recovery after 6 hours exposure to 10°C experimental temperature.

6.4 Discussion

The respiratory response of adult *Thysanoessa inermis* to experimental temperature changes was the same as previously determined (Huenerlage and Buchholz, submitted): increasing temperatures resulted in remarkable metabolic disturbance at temperatures exceeding 12°C. This turning point was determined by the Arrhenius breakpoint temperature (ABT) and consequently, characterized the upper limit of temperature-induced oxygen demand, i.e. the upper pejus temperature limit (TpII; Frederich and Pörtner 2000) after which metabolism changes from aerobic to anaerobic (e.g. Pörtner 2012).

However, the respiratory response does not give a measure for temperature-induced stress on the cellular level. In marine ectotherms, ambient water temperature is one of the major factors causing cellular damages due to denaturation of proteins (e.g. Feder and Hofman 1999, Kültz 2005). Accordingly, species have adapted to respond to environmental stressors by e.g. use of molecular protective chaperones which help to prevent protein degradation and hence, to survive during periods at which the species are exposed to unfavourable abiotic conditions (loc. cit.).

In this framework, we investigated the molecular chaperones 'heat shock protein 70' (Hsp70), which are known as pertinent indicators of cellular stress (loc. cit., Lund et al. 2006, Clark et al. 2011). Five isoforms (B, C1, C2, D and E) were characterized in *T. inermis.* From their structural signatures, they can be divided into categories with different functions and/or locations in the cell (Clark et al. 2011, Cascella 2014, Cascella et al. in prep): constitutive or inducible, cytosolic or mitochondrial Hsp70s. Current analysis of the kinetics of expression will confirm the type of behaviour of these isoforms in response to a temperature at the edge of the metabolic heat tolerance.

Hsp70-B and *Hsp70*-E are usually expressed constitutively, i.e. these isoforms are not inducible by external stress factors (Cascella 2014, Cascella et al. in prep.). In contrast, the other three isoforms would be inducible but located at different regions within the cell. Hsp70-C1 and Hsp70-C2 are located in the intracellular fluid (i.e. cytosolic Hsp70s) whereas Hsp70-D is located in the mitochondria.

In the present study, we found that the 10°C experimental temperature was already sufficient to induce the gene expression of the latter three *Hsp70* isoforms during direct heat exposure as well as during recovery, thus confirming the structural observations. But especially the high level of gene expression during recovery (e.g. up to 80-fold expression in *Hsp70*-C2 compared to the control) is indicative of the molecular repair activities taking place. Isoforms B and E showed no significant response either 3 or 6 hours after shock or during the recovery phase. These kinetics also attested to their strict constitutive state.

Expressions of these different isoforms are compatible with the type of response expected in a species of temperate environments. It is differentiated from the observed responses in other species of krill from other cold environments such as Antarctica. In these euphausiids, the response amplitude is low and nearly absent in short time (Cascella 2014). Comparison of the kinetics of expression revealed differences between inducible isoforms and highlighted the existence of different functionalities and certainly different targets. Thus, the response of

Hsp70 is particularly complex and multifaceted and their study must consider all the different isoforms in their diversity.

However, the current findings imply that *T. inermis* had experienced molecular damage during exposure at 10°C, which is even lower as related to their thermal respiratory limit at 12°C as found by the Arrhenius breakpoint temperature. Therefore, the long-term thermal limit of overall species performance may be < 12°C at least under experimental conditions.

However, to better predict warming effects on the overall performance and population persistence of the arcto-boreal *T. inermis*, further research is needed. The data presented are preliminary only, but give a potential direction for further investigations. In general, the induction of Hsp70 gene expression found, points to the adaptive capacity of *T. inermis* to activate protein protecting chaperones, i.e. to the capability to induce the formation of Hsp70s.

Particularly concerning the Hsp70s and other heat shock proteins, we therefore recommend performing long-term investigations at different temperatures to further elucidate the species potential to acclimate to higher ambient temperatures. Furthermore, It could be interesting to check if the observed decrease of the respiration rate after 12°C can be correlated with the increase or the fall of the expression of the Hsp70. Additionally, other parameters indicating stress will be useful to pin-point the thermal limit of *T. inermis*, like the monitoring of the gene expression of AMP-activated protein kinase (AMPK; Frederich et al. 2008, Jost et al. 2012) or investigations on the accumulation of ROS (reactive oxygen species), antioxidant enzymes (e.g. superoxide dismutases, glutathione S-transferase, catalase or glutathione peroxidase; Tremblay 2014).

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7 SYNOPTIC DISCUSSION AND CONCLUSIONS

The present dissertation gives an insight into the adaptive eco-physiological performance of six krill species from different climatic regions: the subtropical *Euphausia hanseni*, the subtropical-temperate *Nematoscelis megalops*, the boreal *Meganyctiphanes norvegica* and *Thysanoessa longicaudata* and the arcto-boreal *T. raschii* and *T. inermis*. The single species are essential components in their respective ecosystems, being key species in the pelagic food-webs. Furthermore, three of them (*M. norvegica*, *T. inermis* and *T. raschii*) are commercially harvested (Nicol and Endo 1997).

The following discussion relates to the initial questions of the thesis about, I.) the species' specific adaptation to cope with food scarcity, II.) the species' energetic condition within their environments of (recent) origin and III.) their metabolic response to changes in ambient water temperatures. The findings of the single publications (Publications I – V) are aimed at encouraging an increase in the basic physiological knowledge of krill communities from different regions in view of their central function. They may finally offer a basis for complex ecosystem modelling studies under aspects of climate variability (Pörtner and Peck 2010).

7.1 Starvation capacity of krill from two contrasting environments

We investigated the adaptive starvation capacity of the two dominant krill species from contrasting environments: the subtropical-tropical *Euphausia hanseni* from the northern Benguela upwelling region ('Publication I') and the arcto-boreal *Thysanoessa inermis* from the high Arctic Kongsfjord ('Publication II').

The Benguela Current system is a nutritionally poly-pulsed and highly stratified environment (Boyer et al. 2000). Accordingly, krill species from this region rely on seasonal upwelling events which supply phytoplankton patches as food sources. During late austral summer (study period of 'Publication I'), the Benguela upwelling region is characterized by the southernmost extension of the Angola-Benguela Front (at ~ 18°45'S in 2011) and more importantly, minimal upwelling (Hagen et al. 2001a). Zooplankton at the northern boundary of the northern Benguela Current system are therefore subjected to unfavourable trophic conditions, brought about by low nutrient concentrations, and may be exposed to short periods from days to weeks of food deprivation.

In contrast to the Benguela upwelling region, the high Arctic Kongsfjord is a nutritionally mono-pulsed ecosystem with one typical annual spring-bloom (Hop et al. 2002). It is characterized by pronounced seasonal oscillations of the light regime. Accordingly, perpetual darkness and partial sea ice coverage during polar winter lead to complete suppression of pelagic primary production (loc. cit.). As a consequence, herbivorous krill species have to be adapted to a long period of food absence for at least four months.

In general, crustaceans exhibit at least three types of survival strategies to cope with periods of food scarcity. These are defined through different energetic adaptations such as (extreme) metabolic reduction, high storage lipid content and diapause (= 'Type 1'), pronounced reduction in metabolism, use of body reserves and opportunistic feeding (= 'Type 2') or absence of metabolic reduction, combustion of body reserves and opportunistic feeding (= 'Type 3'; Torres et al. 1994). Euphausiids are reported to exhibit a 'Type 2' strategy (Torres et al. 1994, Hagen 1999).

7.1.1 Euphausia hanseni from the northern Benguela upwelling region

The results of 'Publication I' revealed, that the dominant krill species of the northern Benguela Current system, *E. hanseni*, followed the 'Type 1' survival pattern as reported for euphausiids

in general (see above). The specimens responded with a continuous decrease in oxygen consumption by 40 % – 70 % from the first to the last days of starvation. The decline of oxygen consumption rates during starvation was consistent with starvation studies on Antarctic krill *Euphausia superba* (Meyer and Oettl 2005, Auerswald et al. 2009).

Two sub-regions of the northern Benguela Current system were sampled, which were mainly distinguished by different chlorophyll *a* concentrations: the centre of the northern Benguela Current system (NBC) with chlorophyll *a* ≥ 1 mg m^{-3} and the Angola-Benguela Front (ABF) with chlorophyll *a* < 1 mg m^{-3}. In both regions, only seven days of experimental starvation significantly affected the metabolism of the *E. hanseni* specimens.

E. hanseni from the ABF were more sensitive to food deprivation. The oxygen consumption displayed the strongest decrease, which was attributed to the worse nutritional condition of these specimens sampled from this region. Ammonium measurements supported the trophic difference between the NBC and the ABF. The excretion rates in krill from the NBC showed a continuous decrease over the period of starvation, whereas the rates of the specimens from the ABF remained constant from the beginning.

Furthermore, significant differences between regions were found with respect to citrate synthase (CS) efficiency (Michealis-Menten constants K_m). Specimens from the ABF had a significantly higher enzyme efficiency compared to specimens from the NBC. The efficiency of CS provides an indication of the nutritional and metabolic conditions prevailing for organisms sampled from different regions. CS efficiency is known to increase during unfavourable food conditions in order to compensate for metabolic reduction (Buchholz and Saborowski 2000, Saborowski and Buchholz 2002). These findings further supported the assumptions of worse feeding conditions for *E. hanseni* specimens from the ABF and suggest that the specimens from the ABF may already have been exposed to food depletion before capture.

The analysis of elemental body carbon and nitrogen was used as an indicator for the combustion of internal reserves. A preferential use of body carbon (e.g. lipids) to cover energy requirements during starvation was found. This pattern corresponded to findings on adult and larval Antarctic krill (Meyer and Oettl 2005, Auerswald et al. 2009). The seven days of food absence did not alter the total lipid and total protein contents within the *E. hanseni* specimens of either region. This was attributed to the shortness (seven days) of the experiment but also to the very low lipid content exhibited by *E. hanseni*. This species only contained ~ 8 % DW, which is close to the critical minimum of 5 % DW necessary for survival (Hagen et al. 2001b).

In summary, *E. hanseni* adapted quickly to food deprivation by down-regulating metabolic parameters, which helped the species to remain metabolically efficient over the starvation period of seven days. However, the severely low lipid content suggested a limited capacity to overcome longer periods of starvation and highlighted this species' physiological adaptation to the year-round productive Benguela upwelling system with frequent upwelling pulses and only short periods of food absence (Werner and Buchholz submitted).

7.1.2 Thysanoessa inermis from the high Arctic Kongsfjord

In strong contrast to *E. hanseni*, the arcto-boreal *T. inermis*, from the high Arctic Kongsfjord, exhibited a very high total lipid content of up to 50 % DW ('Publication II'). Unlike most other krill species, which predominantly use triacylglycerols as major energy storage (Saether 1986), *T. inermis* stores its lipids in energy-rich wax esters. Wax esters are considered as long-term energy depots and are reported as a major adaptation in polar zooplankton and are commonly found in polar copepods (Scott et al. 2000, Lee et al. 2006). In *T. inermis*, they are

believed to be specifically biosynthesized de novo during the exploitation of the phytoplankton blooms occurring in spring to early summer (Falk-Petersen et al. 2000).

The results of 'Publication II' showed a clear decrease in total lipid in *T. inermis* during the starvation experiment. During the experiment, the metabolic energy demand of this species was mainly covered by the catabolism of storage lipids. The maximum potential survival time of adult *T. inermis* was calculated to be ~ 63 days, which would not be sufficient to cover the metabolic energy requirements of this species assuming a minimum starvation period of 116 days during polar winter (Hop et al. 2002). Accordingly, these results pointed to the presence of additional adaptations used by *T. inermis*, in order to overcome long starvation periods during the winter.

Most surprisingly, however, *T. inermis* did not reduce its overall metabolism during the time of starvation, although this adaptation is believed to be one of the most pronounced responses to starvation in euphausiids and other crustacean zooplankton such as copepods (e.g. Quetin and Ross 1991, Auel et al. 2003, Meyer 2012). During overwintering in *E. superba*, for example, more than 71 % of the energetic costs are covered solely by the reduction of metabolic rates (Quetin and Ross 1991). In contrast, the mean respiration rates of *T. inermis* remained stable during the starvation experiment (~ 6 µmol O_2 h^{-1} gFW^{-1}) and were comparable to unstarved euphausiids investigated at in-situ temperatures from other latitudes (e.g. Ikeda and Mitchell 1982, Saborowski et al. 2002, Auerswald et al. 2009).

Further analyses suggested the overwintering pattern of *T. inermis* as an effective combination of four physiological characteristics: use of internal storage lipids, sexual regression and body shrinkage as common morphological adaptations to reduce metabolic energy demand during times of food scarcity (e.g. Ikeda and Dixon 1982, Meyer 2012) and use of alternative food sources such as occasional predation on calanoid copepods.

The latter was determined from fatty acid analysis in specimens, which had overwintered in-situ. The evaluation of fatty acid trophic markers indicated a significant decrease in diatom markers and the simultaneous increase in *Calanus* markers (Dalsgaard et al. 2003). Calanoid copepods from the Arctic exhibit high lipid contents (e.g. Scott et al. 2000, Auel et al. 2003, Falk-Petersen et al. 2009), and occasional carnivory on these species was proposed to offer *T. inermis* specimens sufficient energy to cover essential energetic requirements.

In conclusion, 'Publication II' revealed that the arcto-boreal *T. inermis* is adapted to successfully cope with long periods of food limitation. However, the species exhibited a different overwintering pattern (i.e. metabolic response to starvation) than krill species from other regions. The survival pattern of *T. inermis* was categorized as an intermediate between the 'Type 1' (linked by its high wax ester content) and the 'Type 3' strategy (linked by its constant metabolic activity and opportunistic feeding behaviour), which is in contrast to the survival patterns investigated in the Antarctic krill *Euphausia superba*, the Northern krill *Meganyctiphanes norvegica* and *E. hanseni* from the Benguela upwelling system (see 'Publication I').

7.2 Energetic condition of five krill species from the Arctic

The investigation of the energetic condition of the krill species studied was especially interesting with regard to the changes in euphausiid composition recently found in the ecosystem of the high Arctic Kongsfjord (Buchholz et al. 2010).

'Publication I' and 'Publication II' highlighted the strong diversity in the lipid contents of two dominant krill species adapted to their specific habitats, i.e. ~ 8 % DW in *E. hanseni* adapted to the northern Benguela upwelling region vs. up to 50 % DW in *T. inermis* adapted to arcto-

boreal environments. Accordingly, species from diverse regions, and new to the Kongsfjord ecosystem, may carry different lipid contents of varying composition to the fjord and alter the trophic environment at higher trophic levels. Furthermore, due to possible adjustments in feeding mode, the predator pressure on lower trophic levels, such as small zooplankton and phytoplankton, may change.

'Publication III' tackled such questions by analysing total lipid content, lipid class and fatty acid composition of the five krill species from different climate zones: the subtropical-temperate *N. megalops*, the boreal *M. norvegica* and *T. longicaudata* and the arcto-boreal *T. raschii* and *T. inermis* (Buchholz et al. 2010).

Significant interspecific differences between the five krill species were found. The species did not only differ in their total lipid content, but also in their intrinsic lipid composition and specific trophic ecology.

The arcto-boreal *T. inermis*, dominating the Kongsfjord ecosystem exhibited the highest mean lipid contents (35 – 54 % DW) mainly stored as energy-rich wax esters (41 – 55 % of total lipid; see also 'Publication II'). This characteristic makes *T. inermis* one of the most important food sources for its predators. It is believed to determine the recruitment success, individual growth and overall population condition of economically important North Atlantic fish species such as blue whiting (*Micromesistius poutassou*), herring (*Clupea harengus*), capelin (*Mallotus villosus*), haddock (*Melanogrammus aeglefinus)*, juvenile polar (*Boreogadus saida*) and Atlantic cod (*Gadus morhua*) (Gjøsæter et al. 2002, Dalpadado and Bogstad 2004, Dolgov et al. 2010, Dalpadado and Mowbray 2013).

The results in 'Publication III' revealed that the total lipid content of the boreal *T. longicaudata* was similar to that of the arcto-boreal *T. inermis*, whereas the lipid content of the arcto-boreal *T. raschii*, the boreal *M. norvegica* and the subtropical-temperate *N. megalops* were significantly lower (~ 30 % and ~ 10 % DW). Due to the differences in lipid content, these species were proposed as a lower quality food item to higher trophic levels than *T. inermis*, whereas the very low lipid content of *N. megalops* will be of the lowest nutritional quality to the Arctic predators in Kongsfjorden relying on lipid rich nutrition.

Comparable to *E. hanseni* from the northern Benguela upwelling area ('Publication I'), *N. megalops* also appeared to be adapted to environments with no or only short periods of food absence requiring only low or almost no lipid storage. However, its broad spatial distribution (Fig. 1 in the introduction of this thesis), suggests different physiological adaptations, enabling this species to survive in contrasting environments, e.g. in the Benguela Current system, the Mediterranean Sea, the temperate North Atlantic and in subarctic regions (Gopalakrishnan 1974a, Zhukova et al. 2009, Buchholz et al. 2010).

Both the fatty acid trophic marker signals and the stable isotope studies suggested *N. megalops* as a predominantly carnivorous species. The major fatty acids were the 18:1(n-9) fatty acid and the 20:1(n-9/n-7) and 22:1(n-11/n-9) fatty acids, which are typical trophic markers for carnivory and the ingestion of calanoid copepods, respectively (Graeve et al. 1997, Dalsgaard et al. 2003, Legezynska et al. 2014). In contrast, diatom markers (Stübing and Hagen 2003) only accounted for ~ 7 % of the total fatty acids.

The preference for carnivory in *N. megalops* was also reported in other studies, deduced from its morphological appearance [lacking a feeding basket and having extended thoracic appendages most likely assisting predation (Gopalakrishnan 1974b, Mauchline et al. 1989)] as well as gut content, stable isotope and fatty acid analyses (Gurney et al. 2001, Barange et al. 1991, Mayzaud 2007, Cartes 2011). Accordingly, its carnivorous preference may form a trophic niche for *N. megalops* during habitat competition with other krill species and sustains survival when phytoplankton is scarce or even absent.

The results in 'Publication III' further highlighted the carnivorous feeding pattern in the boreal *M. norvegica*. Stable isotope analyses pointed to a generally omnivorous feeding habit, whereas fatty acid analyses showed the highest amount of fatty acids originating from calanoid copepods (~ 30 % of the total fatty acids). This opportunistic omnivorous feeding mode with carnivorous tendency was also reported for *M. norvegica* sampled in North Atlantic waters (e.g. Falk-Petersen et al. 2000, Kaartvedt et al. 2002, Petursdottir et al. 2008). The present study on the *Thysanoessa* species pointed to a higher tendency to a more herbivorous feeding than in the previously discussed subtropical-boreal *N. megalops* and the boreal *M. norvegica*. This was substantiated by multivariate statistics and was in accordance with other studies conducted on *Thysanoessa* species (e.g. Falk-Petersen et al. 1981). Both *T. raschii* and *T. longicaudata* showed a higher preference for carnivorous feeding than *T. inermis* as these species contained a fatty acid trophic marker, which most probably originated from carnivory on other metazoans (e.g. Falk-Petersen et al. 2000).

In summary, not only the nutritional value but also the feeding preferences of the different krill species may alter the ecosystem balance of the high Arctic Kongsfjord. Both the boreal euphausiids *M. norvegica* and *T. longicaudata* and the subtropical-boreal krill *N. megalops* may be prospective species of increasing trophic relevance within the marine food-web of the Kongsfjord. Top predators relying on krill as a food source will be exposed to a new diet of most probably lower quality. Furthermore, increasing abundances of the above mentioned krill species may result in stronger predation pressure on lower trophic levels (e.g. phytoplankton, copepods, fish eggs and fish larvae) and, concomitantly, increase the competition on shared food sources with other macro-and mesozooplankton. The increased abundances of the predominantly carnivorous euphausiids *M. norvegica* and *N. megalops*, may lead to considerable interspecific competition with juvenile planktivorous fish. This has been reported for *M. norvegica* from other study areas, in which this species was observed to be one of the major predators of calanoid copepods in Oslofjord, Southern Norway (Beyer 1992). Additionally, owing to its large potential body length of up to 50 mm, *M. norvegica* may itself be a direct predator on fish larvae and small euphausiids (Tarling et al. 2010).

7.2.1 Energy storage patterns

Both 'Publication II' and 'Publication III' highlighted the exceptional dominance of wax esters in the storage lipids of *T. inermis*. Wax esters are a long-term energy store and a valuable energy source during times of food scarcity (Scott et al. 2000, Falk-Petersen et al. 2000, Lee et al. 2006). Apart from *T. inermis*, wax ester dominated storages have only been found in some euphausiid species such as the Antarctic *Euphausia crystallorophias* and *Thysanoessa macrura* (Falk-Petersen et al. 2000). 'Publication III' aimed to investigate the lipid class composition of the krill species recently introduced to the high Arctic Kongsfjord as it may be used as an indication for the adaptive capacity to survive times of food scarcity.

The lipids of the subtropical-temperate *N. megalops* mainly consisted of polar lipids, which have no storage function at all. Thus it was assumed that the species was unable to survive long starvation periods. However, as a predominantly carnivorous species (see above) it may feed on lipid rich (diapausing) copepods to overwinter.

In contrast, the major storage lipid of *T. raschii*, *T. longicaudata* and *M. norvegica* were triacylglycerols (47 %, 52 % and 68 % respectively). Triacylglycerols are primarily accumulated in krill and other zooplankton species from lower latitudes, which do not have to cope with long starvation periods as found in polar regions (e.g. Clarke 1980, Lee et al. 2006). Nevertheless, the lipid stores of the polar endemic Antarctic krill species *Euphausia*

superba are also dominated by triacylglycerols (e.g. Meyer 2012). In contrast to wax esters, triacylglycerols are known to serve as short-term energy stores, which are rapidly catabolised if energy is required (Lee et al. 2006). Accordingly, to survive during polar winter, *E. superba* reduces its overall metabolism and uses alternative food sources (Meyer 2012).

In summary, both the boreal species *M. norvegica* and *T. longicaudata* and the arcto-boreal species *T. raschii* may switch to alternative food sources and/or metabolism reducing adaptations similar to *E. superba*. This was supported by earlier studies which found that the arcto-boreal *T. raschii* feeds on sediments and detritus over winter to cover energy requirements (Berkes 1976, Sargent and Falk-Petersen 1981, Schmidt 2010). Epibenthic and benthic feeding was also observed in *M. norvegica* (Greene et al. 1988, Schmidt 2010). Additionally, being opportunistically omnivorous (Mauchline 1980), *M. norvegica* may share the pattern suggested for *N. megalops* and feed on lipid rich copepods to overwinter.

7.3 Metabolic response to temperature changes

In ectotherms, temperature is the major factor determining an organism's overall metabolic performance and hence, survival and biogeographic population persistence (e.g. Pörtner 2001, Pörtner and Peck 2010). In order to better predict the effects of climate variability, the study on species-specific metabolic reaction to temperature changes is therefore relevant to assess the fate of biological communities, their changes in distribution patterns and thus, ecosystem functioning (Somero 2005).

Temperature controlled experiments are common approaches to investigate the temperature windows of different species (Pörtner 2002). Respiration rate measurements are often used as a proxy for the sum of physiological processes, i.e. to provide a measure for cellular metabolic energy (ATP) provision and demand and hence, species-specific overall metabolism (Williams and Del Giorgio 2005).

'Publication I' investigated the temperature-induced routine metabolic rates of the subtropical-tropical krill *E. hanseni* from the northern Benguela Current system, and 'Publication IV' the thermal sensitivity of four krill species from different origins currently found in the high Arctic Kongsfjord: *T. inermis*, *T. raschii*, *M. norvegica* and *N. megalops*. 'Publication IV' aimed to uncover potential thermal limits, which may explain these species' recent changes in biogeographic distribution and/or the species' fate during passive transport, i.e. during (future) changes in distribution.

Both publications, 'Publications I' and 'Publication IV', compared the species' specific metabolic adaptation to their environments of origin: the subtropical-tropical *E. hanseni*, the subtropical-temperate *N. megalops* and the boreal *M. norvegica* appeared to be adapted to strong temperature changes (4 – 15°C and 0 – 16°C), whereas the results of 'Publication IV' and 'Publication V' revealed a respiratory thermal limit for the arcto-boreal *Thysanoessa* species.

Euphausia hanseni from the northern Benguela upwelling region

'Publication I' emphasized the importance of one of the most abundant krill species from the northern Benguela upwelling system, *E. hanseni*, and its energetic adaptation to this highly productive environment, i.e. its low starvation capacity. The study also involved investigation into the metabolic response to temperature changes as *E. hanseni* is known to perform extensive diel vertical migration during which it encounters a wide range of ambient temperatures (Barange 1990, Barange and Stuart 1991, Barange and Pillar 1992, Werner and Buchholz 2013).

The study revealed a strong relationship between respiration rate and temperature. The rates increased exponentially with increasing temperatures following the Q_{10} rule (van't Hoff 1884) and were in agreement with previous studies on krill species of other latitudes (e.g. Saborowski et al. 2002).

Accordingly, *E. hanseni* showed the adaptive capacity to physiologically compensate for the temperature-induced changes in oxygen demand, which is pivotal when the species enters warm surface waters for feeding (Werner and Buchholz 2013).

7.3.1 Krill from the high Arctic Kongsfjord

'Publication III' highlighted the strong differences in the lipid composition of the krill species recently found at high latitudes, which may have remarkable impact on the ecosystem's food-web in the future.

Physiologically it is not yet proven if the krill species, which currently form the euphausiid community of the Kongsfjord, can thrive and persist at this high latitude. Several projections point to potential climatic warming shifts, which may further improve conditions for the expatriate species of Atlantic origin (Falk-Petersen et al. 2007). Attributed to climate change, the Arctic ecosystem is in a transition to a warmer state (Polyakov et al. 2007). Recently, the input of warm Atlantic water masses increased, enhanced by exceptional warm winters and a resulting decrease in winter sea ice coverage.

The aim of the study for 'Publication IV' was therefore to determine the metabolic response of four krill species from different origins (the arcto-boreal *Thysanoessa inermis*, the arcto-boreal *T. raschii*, the boreal *Meganyctiphanes norvegica* and the subtropical-temperate *Nematoscelis megalops*) to ambient temperature changes in order to distinguish potential limits which may determine their success in biogeographic distribution. It further investigated the species' overall physiological constitution during late summer and early spring.

All specimens sampled were in good condition. Analysis of stomach fullness indicated active feeding in all specimens from both seasons sampled. As in *E. hanseni* ('Publication I'), the temperature experiments on the respiration rates of both the temperate-boreal *M. norvegica* and the subtropical-temperate *N. megalops* revealed that these species were able to compensate for associated changes in oxygen, i.e. energy demand. Respiration rates were positively correlated to temperature and showed a characteristic Q_{10} relationship computed for marine zooplankton over the investigated temperature range (Ikeda 1985, Ivleva 1980). The metabolic reactions to temperature were similar to previous investigations performed at lower latitudes (e.g. Saborowski et al. 2002, Werner et al. 2012), which indicated that temperature acclimatization did not seem to play a significant role within both krill species (Clarke 1991). Accordingly, the specimens were not yet exposed to their specific upper or lower respiratory pejus temperatures, which highlights the considerable metabolic plasticity of these species. These findings may explain the good metabolic performance of both *M. norvegica* and *N. megalops* during passive transport, which is further supported by observations of increasing abundances of these species at higher latitudes (Dalpadado et al. 2008, Zhukova et al. 2009, Buchholz et al. 2010). However, it remains unclear whether the subtropical-temperate *N. megalops* is sufficiently adapted to temperatures < 4°C. Due to technical reasons these temperatures were not covered in the present experiments. In comparison to the temperate-boreal *M. norvegica*, a potential low pejus temperature between 0 and 4°C in *N. megalops* may affect this species' survival and concomitantly, implicate population failure during further northward expansion.

In contrast to *M. norvegica* and *N. megalops*, the overall metabolic reaction to increasing temperatures in the two arcto-boreal krill species, *T. inermis* and *T. raschii*, was remarkably

different. An acute increase of experimental temperatures resulted in considerable respiratory disturbance by 12°C. From this point, defined as the Arrhenius breakpoint temperature (ABT), respiration rates levelled out and became independent of further temperature increase. The ABT probably indicated the beginning of pejus (Latin = worse) hemolymph oxygenation and was therefore equated to these species' upper pejus temperature limit (TpII; Frederich and Pörtner 2000).

However, metabolic response to short-term induced temperature changes may only reflect an individual's passive survival strategy and hence, time-limited tolerance. As the specific long-term thermal tolerance of an organism results from the complex interaction of small scale constituents (e.g. molecules, organelles, cells and tissues; Pörtner 2002, Pörtner and Peck 2010, Somero 2010), 'Publication V' aimed to further investigate the thermal limit found in the *Thysanoessa* species on a molecular level. In marine ectotherms, ambient water temperature is one of the major factors causing cellular damage due to the denaturation of proteins (e.g. Feder and Hofman 1999, Kültz 2005). Accordingly, species have adapted to respond to environmental stressors by e.g. use of molecular protective chaperones, which help to prevent protein degradation and hence, to survive during periods at which the species are exposed to unfavourable abiotic conditions (loc. cit.).

'Publication V' investigated the temperature induced gene expression of five isoforms of the molecular chaperone 'heat shock protein 70' (Hsp70). The results revealed that an experimental temperature of 10°C was sufficient to induce the gene expression of the three *Hsp70* isoforms during direct heat exposure as well as during recovery. *Hsp70*-C2 showed an up to 80-fold expression during recovery compared to the control. This finding revealed that *T. inermis* had experienced molecular damage during exposure at 10°C, which is lower than their thermal respiratory limit at 12°C as determined by the Arrhenius breakpoint temperature ('Publication IV' and 'Publication V'). Therefore under experimental conditions, the long-term thermal limit of overall species performance may be < 12°C.

In summary, the findings of 'Publication IV' and 'Publication V' offered a possible explanation of why *Thysanoessa* spp. populations diminished at lower latitudes such as in the Newfoundland and Labrador shelf ecosystem. In this area, warming anomalies sometimes exceeded the upper metabolic pejus temperatures suggested for the *Thysanoessa* species (summer surface temperatures of up to 15°C; Colbourne et al. 2013). Accordingly, consistent increase of ambient water temperature may have led to a loss of metabolic performance in both species and hence, may have altered the long-term population persistence of *T. inermis* and *T. raschii* within this region or at lower latitudes, respectively. Furthermore, the findings highlighted these species' strict arcto-boreal biogeographic distribution associated to colder water temperatures.

In comparison, the metabolic plasticity of the temperate-boreal *M. norvegica* and subtropical-temperate *N. megalops* supported the increase in abundance of these species at lower latitudes. As in the subtropical-tropical *E. hanseni* from the Benguela Current system, the temperature-induced respiration curves indicated that both the latter species are adapted to strong temperature fluctuations irrespective of sampling region and latitude. Furthermore, due to its nutritional variability ('Publication III'), superior overall performance and high metabolic plasticity ('Publication IV'), the temperate-boreal *M. norvegica* may profit from the future projections of the high Arctic Kongsfjord ecosystem in transition to a warmer state. Also the *Thysanoessa* species may, at first sight, benefit due to increased water temperatures favourable for reproduction (Buchholz et al. 2012). In contrast, Arctic climate warming may negatively affect the overall metabolic performance and thus, regional persistence of the *Thysanoessa* species as they may experience maximum water temperatures close to their thermal limit.

7.4 Perspectives

The data collected in this dissertation may be useful as a basis for ecological modelling especially focussing on climate interactions and dynamic coupling of food-webs (Pörtner and Peck 2010). Part of the respiration data were already integrated into a general euphausiid respiration model, which aimed to analyse seasonal respiration patterns within single euphausiid species in due consideration of sampling latitude, sampling period and number of daylight hours (Tremblay et al. 2014; see Appendix 'II. Co-Authored ISI Peer-Reviewed Publications'). Furthermore, the oxygen consumption rates, as a proxy for carbon demand, the lipid compositions and stable isotope ratios of the different species from the high Arctic Kongsfjord (i.e. the data from 'Publication II' – Publication IV') are planned to be incorporated into a complex food-web analysis, which is in preparation for the Kongsfjord ecosystem (Asmus H. and Paar M., AWI Sylt, pers.comm.).

The findings of the present thesis underlined the role of euphausiids as key components in marine ecosystems. The results emphasized the need for further research on the various species' physiology. Scientific knowledge on most euphausiids is still minor, although this is vital to assess the status of the diverse krill stocks in ecosystem functioning and for effective management of related fisheries. The commercial demand for krill products will probably increase in the near future, which may have an impact on various species and increase krill fisheries in near shore waters (Nicol and Endo 1997). Accordingly, the ecophysiological comparison with other krill species will extend the applicability of krill as a biological indicator in ecosystem analysis.

The conclusions of the single publications ('Publication I' – 'Publication V') lead to suggestions for experiments and measurements, which could be included in future research, based on their respective results.

Further investigations may include experiments on the maximum starvation capacity of *E. hanseni* from the northern Benguela upwelling system ('Publication I'), i.e. the determination of the "point-of-no-return" by adding re-feeding experiments to the protocol. Temperature experiments on the overall metabolic rates of this species should be extended, in order to investigate its upper and lower thermal limits.

Respiratory thermal limits of krill species, as a proxy for animal metabolic performance, are hardly known, although these form the physiological basis for understanding mechanistic biogeographic distribution patterns of species (Pörtner and Farrell 2008). Concomitantly, comparative experiments would be feasible for the subtropical-temperate *N. megalops*, the boreal *M. norvegica* and the mainly boreal *T. longicaudata* recently found in the high Arctic Kongsfjord ('Publication IV').

The results of 'Publication IV' and 'Publication V' revealed the upper respiratory thermal limit of the two arcto-boreal *Thysanoessa* species *T. inermis* and *T. raschii*. However, to better predict warming effects on the overall performance and population persistence of these species, further research is needed. As examples, this may include investigations on the accumulation of anaerobic metabolites (such as lactate and succinate) and reactive oxygen species (ROS) and respectively, the specific activity of antioxidant enzymes (e.g. superoxide dismutases, glutathione S-transferase, catalase or glutathione peroxidase; Tremblay 2014). Additionally, different molecular parameters may be useful to further investigate the thermal adaptive capacity of these species, like the gene expression of metabolic enzymes such as of the AMP-activated protein kinase (AMPK; Frederich et al. 2008, Jost et al. 2012).

In future experiments, molecular approaches may also be useful to further investigate the (energy) metabolism of the species investigated. As physiological mechanisms in krill may be

regulated by synchronization with the seasonal changes in polar light regime these approaches may include investigating the function of endogenous biological clock genes (Teschke et al. 2011, Tessmar-Raible et al. 2011). Furthermore, the energy storage of *T. inermis* may be of special interest ('Publication II' and 'Publication III'). It was the only species that extensively stored wax esters, which were synthesized de novo and accumulated in multiple lipid vesicles forming a saddle-like structure located in the species' cephalothorax (Falk-Petersen et al. 2000, Buchholz et al. 2010). Accordingly, the molecular investigation on related enzymes for wax ester formation may be useful to closer investigate season-induced differences for lipid accumulation (such as the gene expression of fatty acyl desaturase and elongase genes; e.g. Morais et al. 2011). The detailed lipid accumulation may also be followed by feeding experiments with labelled food sources (Henderson et al. 1982).

Most parts of the thesis concerned the metabolic and energetic performance of the five different krill species from the high Arctic Kongsfjord ('Publication II' – 'Publication V'). The scientific findings of these publications underlined the future possible changes in the food-web of this ecosystem. The Arctic in general was reported in a transition to a warmer state (e.g. Polyakov et al. 2007, Spielhagen et al. 2011), which may in the future enhance the living conditions for species originating from lower latitudes and hence, imply changes in different krill species' persistence.

The differences between the five species were particularly pronounced with regard to the energetic conditions and the overall metabolic performance. Accordingly, in order to best predict possible future (food-web) implications, the 'Publication III' – 'Publication V' underline the need for further investigation on the single krill species' population dynamics. During the last decades, investigations indicated an annual increasing abundance of the krill species originating from lower latitudes (e.g. Zhukova et al. 2009, Buchholz et al. 2010, Eriksen and Dalpadado 2011, McBride et al. 2014). According to the findings of 'Publication III' this may, in the future, have considerable impacts on the higher trophic levels due to the low lipid contents found in the krill species concerned. On the other hand, the increased biomass of all the euphausiid species in general, including the biomass of *T. inermis*, may compensate for the lower nutritional quality of the single specimens and therefore, this scenario may be beneficial for the predators of krill in general (Dalpadado et al. 2012).

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APPENDIX

This chapter presents electronic supplementary material (ESM), which were not included in the publications summarized in this thesis.

A1 ESM to publication II

In: *Huenerlage et al. (2014)* Lipid composition and trophic relationships of krill species in a high Arctic fjord. Polar Biology S.I. Kongsfjord (DOI: 10.1007/s00300-014-1607-6)

Online resource 1 *Lipid class composition (n = number of individuals) of Thysanoessa inermis (Ti KF), T. raschii (Tr), T. longicaudata (TL), Meganyctiphanes norvegica (Mn) and Nematoscelis megalops (Nm) and of T. inermis sampled in Hornsund (Ti HS). Values are given as mean percentages ± standard deviations.*

Lipid classes	*Ti* HS (n = 7)	*Ti* KF (n = 13)	*TL* (n = 3)	*Tr* (n = 3)	*Mn* (n = 3)	*Nm* (n = 4)
	(% total lipids)					
Wax ester	46.2±4.5	46.7±1.7	5.3±2.0	2.6±1.3	-	8.9±2.3
Triacylglycerols	21.3±0.8	17.2±4.8	51.8±1.4	47.3±9.6	67.5±2.7	5.8±2.4
Sterols	1.1±0.5	0.9±1.3	1.9±0.8	1.4±1.3	2.6±0.5	13.1±0.7
Free fatty acids	1.6±0.9	0.8±0.3	1.1±0.6	1.3±0.5	1.5±0.2	tr
	3.1±0.7	3.1±1.0	3.6±0.1	2.1±1.6	6.0±0.8	26.3±2.8
Phosphatidylcholine	31.7±0.1	28.7±6.4	40.3±1.2	45.3±5.6	22.4±1.5	47.4±2.2

A2 ESMs to publication IV

In: *Huenerlage & Buchholz (2015)* Thermal limits of krill species from the high Arctic Kongsfjord (Svalbard). Marine Ecology Progress Series, in press (DOI: 10.3354/meps11408)

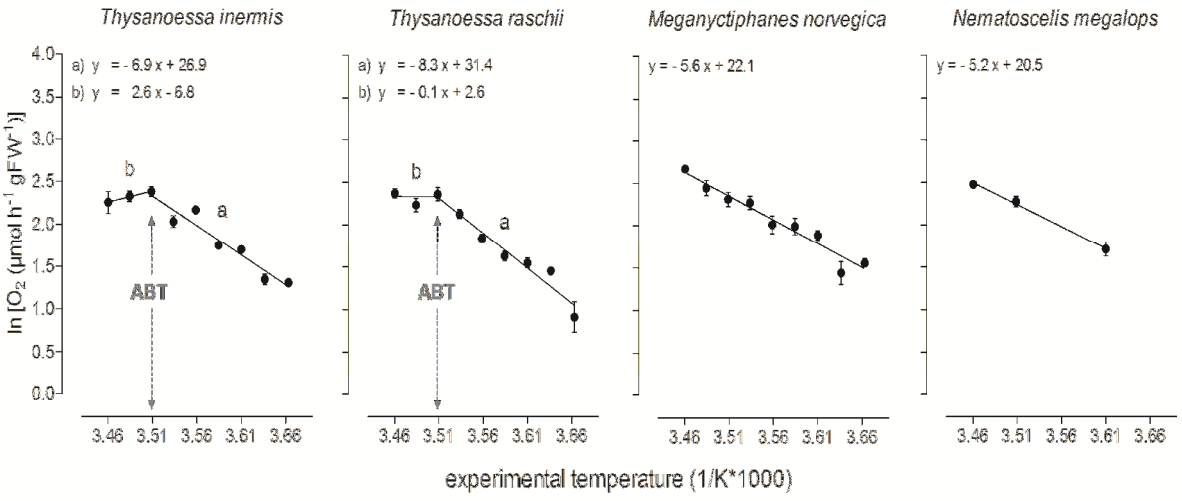

Fig. S1 *Arrhenius plots of normalized respiration rates of adult Thysanoessa inermis (n = 20 – 35; n = number of individuals used in the temperature experiments), T. raschii (n = 5 – 9), Meganyctiphanes norvegica (n = 5 – 19) and Nematoscelis megalops (n = 2 – 4) in relation to experimental temperature. Lines show linear regressions. Regression equations are given in each graph. ABT = Arrhenius breakpoint temperature defined by a significant change in slope. Values are given as means ± SEM (if not visible, SEM are covered by the symbol)*

Table S1. *Respiration rates (A), excretion rates (B) and O:N-ratios (C) of adult Thysanoessa inermis (n = 20 – 35). T. raschii (n = 5 – 9). Meganyctiphanes norvegica (n = 5 – 19) and Nematoscelis megalops (n = 3 – 4) in relation to experimental temperature. Values are given as means ± SEM.*

A) Respiration rate (µmol O_2 h^{-1} gFW^{-1})		0	2	4	6	8	10	12	14	16
T. inermis		3.8 ± 0.2	4.0 ± 0.2	5.5 ± 0.3	5.9 ± 0.3	8.8 ± 0.2	8.1 ± 0.5	11.2 ± 0.7	10.7 ± 0.6	10.6 ± 0.9
T. raschii		2.7 ± 0.5	4.3 ± 0.2	4.7 ± 0.3	5.1 ± 0.3	6.3 ± 0.3	8.4 ± 0.5	10.9 ± 1.0	9.5 ± 0.8	10.8 ± 0.6
M. norvegica		4.8 ± 0.2	4.6 ± 0.8	6.8 ± 0.4	7.5 ± 0.7	7.9 ± 0.8	9.8 ± 0.8	10.4 ± 0.9	11.9 ± 0.9	14.6 ± 0.8
N. megalops		-	-	5.6 ± 0.4	-	-	-	9.8 ± 0.6	-	11.9 ± 0.0
B) Excretion rate (µmol NH_4-N h^{-1} gFW^{-1})		**0**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
T. inermis		0.3 ± 0.0	0.3 ± 0.0	0.7 ± 0.0	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	1.4 ± 0.2	1.2 ± 0.1	1.8 ± 0.2
T. raschii		0.8 ± 0.2	1.2 ± 0.4	1.0 ± 0.3	0.9 ± 0.3	1.6 ± 0.4	1.5 ± 0.3	2.4 ± 0.8	2.3 ± 0.6	2.3 ± 0.5
M. norvegica	Autumn	0.3 ± 0.0	0.2 ± 0.1	0.4 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.7 ± 0.3	0.9 ± 0.1	0.6 ± 0.1	1.1 ± 0.4
	Spring	-	0.5 ± 0.1	0.8 ± 0.0	-	1.4 ± 0.2	-	1.7 ± 0.3	-	3.8 ± 1.0
N. megalops		-	-	0.6 ± 0.1	-	-	-	1.4 ± 0.2	-	2.4 ± 0.4
C) O:N- ratio		**0**	**2**	**4**	**6**	**8**	**10**	**12**	**14**	**16**
T. inermis		32.5 ± 3.7	33.0 ± 4.1	24.3 ± 1.5	23.3 ± 1.7	22.5 ± 1.6	26.6 ± 4.3	21.4 ± 2.8	18.0 ± 1.1	12.7 ± 1.9
T. raschii		13.8 ± 3.0	19.3 ± 2.7	24.1 ± 3.4	17.8 ± 2.2	14.9 ± 2.3	20.2 ± 3.1	21.3 ± 2.8	4.8 ± 1.2	8.6 ± 2.2
M. norvegica	Autumn	31.2 ± 5.6	28.0 ± 6.8	38.3 ± 4.9	34.2 ± 6.8	30.0 ± 9.5	39.5 ± 13.1	26.0 ± 3.0	43.9 ± 5.9	43.0 ± 13.0
	Spring	-	11.4 ± 1.0	17.1 ± 1.3	-	11.8 ± 1.4	-	14.7 ± 2.9	-	9.8 ± 2.5
N. megalops		-	-	13.5 ± 7.5	-	-	-	12.8 ± 3.3	-	15.5 ± 2.2

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